# JOURNAL OF DAIRY SCIENCE

NOVEMBER, 1957

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Estrus Expression in Cattle
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Nitrogen in Calcium Caseinate–Phosphate Complex
Homogenization of Ultrasonically Irradiated Milk
Heat Tolerances of Jersey and Sindhi Crossbreeds
Call for Papers for the 1958 Annual Meeting of the American Dairy Science Association

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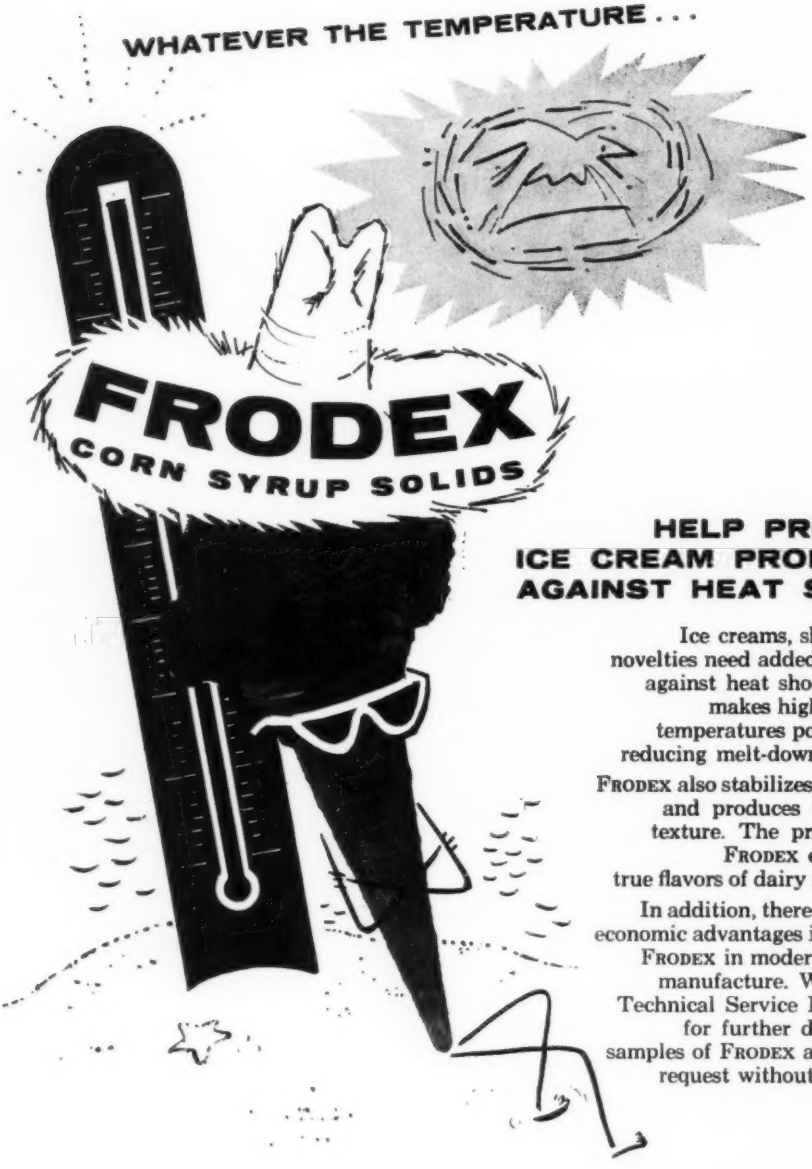


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# PEOPLE and EVENTS
## in the Dairy Science World

### Call for Papers for the 1958 Annual Meeting of the American Dairy Science Association

The 53rd annual meeting of the American Dairy Science Association will be held June 17, 18, and 19, 1958, at the North Carolina State College, Raleigh.

Members who wish to present papers must furnish titles and abstracts not later than February 1. This deadline must be met so that the complete program may appear in the Journal for May and so that the abstracts may be printed for distribution at the annual meeting. Abstracts must contain not over 200 words—arrived at by counting each typed line as 12 words. Abstracts longer than 200 words will be returned to the authors by the Committee.

An original and three copies of each abstract should be prepared. The original and one copy is to be mailed to the Chairman, another copy to the Vice-Chairman, and a fourth copy to the Secretary of the Section before which the paper is to be presented.

The title should clearly indicate the nature of the research reported. Complete data should be presented showing the major results of the research. Essential but not excess statistics will make the data more understandable. The recommended Journal of Dairy Science abbreviations should be used. Also, refer to the October, 1957, issue of the *Journal of Dairy Science* for detailed suggestions for abstract or summary preparation.

All members of the Association are encouraged to present papers. Participation by members in the Industry and by senior members of the Association are especially welcome. Abstracts of research should not be submitted if articles using the same data have been accepted by a scientific journal for publication prior to the annual meeting. In years past the Program Committee has favored the general policy that an individual be limited to presentation of one paper or appear as an author on not more than two papers. With reservations the majority of the membership accepted this policy recognizing that it limits or restricts the reporting of data by full-time researchers and by departments devoting a major proportion of their time to research. Your Program Committee along with you desires (1) to stimulate vigorous and sound research, and (2) to continue to improve the quality of the papers presented; furthermore, they sincerely believe that reporting scientific results on a friendly, yet competitive basis is the life of our organization. This year your Program Committee is liberalizing past policy by permitting an individual to submit the number of papers he desires, provided that if he exceeds the number stated in the present policy his *department* must rate the abstracts in order of current importance, value, and contribution to Dairy Science. The Program Committee reserves the privilege to reduce the number of papers submitted by an individual if, after receiving all papers, it is felt that others should be submitted. The papers not selected for presentation will be read by title only.

Members prefer to receive mimeographed copies of pertinent material which must be presented visually (minimum 250 copies); such material includes tables of data, graphs, and brief summaries. Material for projection on screens should be used only if the preferred method is impossible.

Names and addresses of officers of sections to whom titles and abstracts will be sent are:

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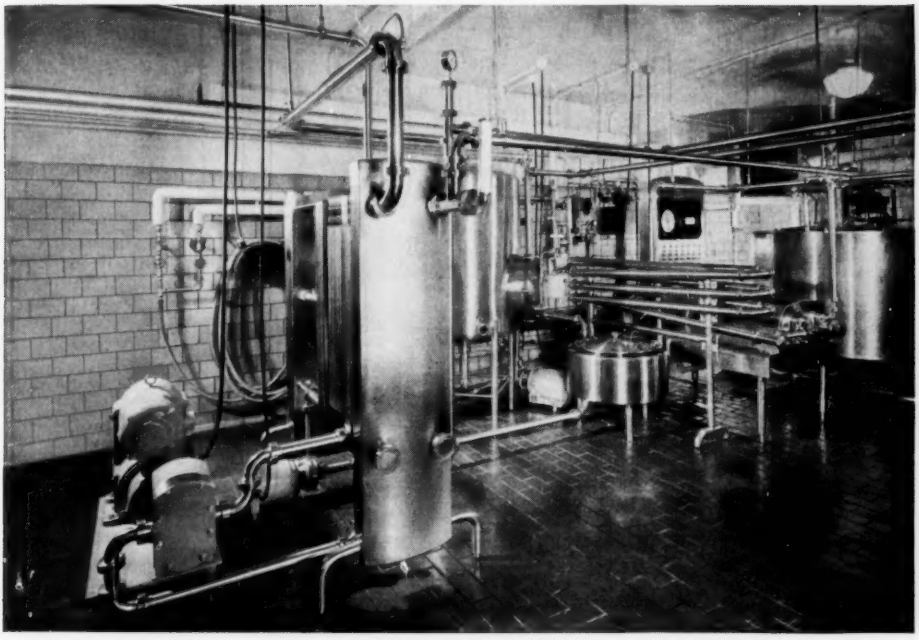

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## Distinguished Canadian Dairy Scientist Retires

W. H. SPROULE retired in August of this year as Head of the Dept. of Dairy Science of the Ontario Agricultural College, Guelph, a position he held for twenty-five years.

Professor Sproule was born at Vankleek Hill in Ontario in 1894. He entered the Ontario Agricultural College in 1912 but left to join the Royal Flying Corps (World War I), resuming his studies after the war, graduating from the O.A.C. in 1920, at which time he was appointed to the Staff of the Department of Dairy Science. He succeeded H. H. DEAN as head in 1932. Prof. Sproule did postgraduate work at the Univ. of Wisconsin, Madison.

**W. H. Sproule**

The contributions of Professor Sproule to Canadian Dairying are many and varied. He organized the Ontario Dairy Research Council; worked on the application of wide curd knives for cheesemaking; organized cheese sediment testing in Ontario, and inaugurated the yeast and mold service for Ontario creameries. He was author and co-author of a number of scientific papers and bulletins, including "Standardization and Neutralization of Acidity of Churning Cream"; "The Mineral and Vitamin Components of Ontario Cheese and Whey"; "The Development of Canadian Blue Cheese"; "The Vitamin A Content of Ontario Butter"; "Effect of Cooking on Cheddar Cheese"; "Yields of Cheddar Cheese," and "Low-fat Cheddar Cheese."

Prof. Sproule was an active member of the American Dairy Science Association, served on many A.D.S.A. committees, and was host to the A.D.S.A. meetings in 1947. An Ontario Dairy Industry delegate to the Thirteenth International Dairy Congress at The Hague in 1953 and an Ontario Dept. of Agriculture delegate to the Fourteenth International Dairy Congress at Rome in 1956.

Professoar Sproule's main contribution was as a teacher and thousands of students have come under his influence.—D. M. IRVINE.

## Univ. of Florida News

R. A. EASTWOOD has been appointed Marketing Specialist in Dairy and Poultry Products with the University of Florida Agricultural Extension Service, Gainesville. A native of Wyoming and graduate of the University of Wyoming, he was an assistant county agent; served two years during Word War II, and holds M.S. and Ph.D. degrees from Cornell University, Ithaca, N. Y. For the past three years he directed the Department of Affiliated Unit Service of the National Dairy Council. He came to the Florida position during the summer of 1957.

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The 22nd Annual Dairy Field Day at the University of Florida Dairy Research Unit was attended by some 200 dairymen and families on August 1-2, 1957. The program included discussions of pasture irrigation, WADAM record keeping and the milk-o-meter, large animal diagnostic laboratory, frozen semen, and a report by the PDCA.

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Thirty herd owners and herdsmen attended the Annual Dairy Herdsmen's Short Course on August 20-23, 1957. The program dealt with phases of reproduction, rumen digestion with relation to feeding, calf raising, herd building, mastitis, reproductive diseases, dairy farm machinery and its maintenance.

## Briggs Heads New Carnation Division

J. A. BRIGGS has been named to the newly-created post of national manager of the Carnation Company Pet Food Division.

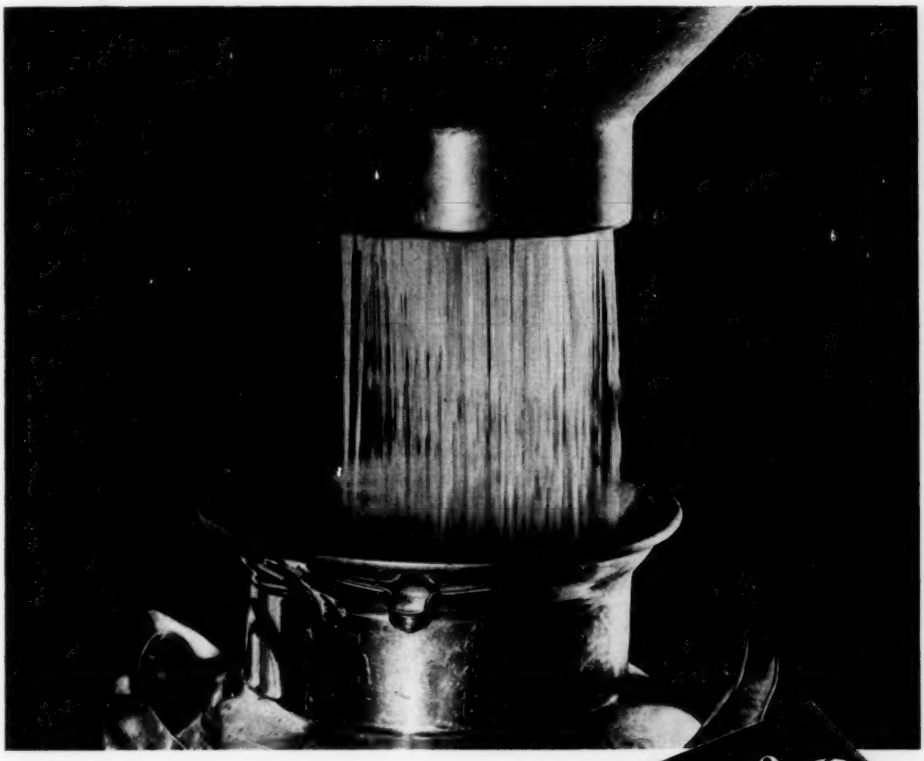
A graduate of Stanford Univ., Palo Alto, Calif., Briggs was first employed as advertising director for the Los Angeles *Downtown Shopping News*. He then served as advertising manager of the W. K. Kellogg Company, Battle Creek, Mich.

## Ghormley Feted by Dairy Shrine Club

ALFRED M. GHORMLEY (left), president of Carnation Company, was named Guest of Honor for 1957 by the Dairy Shrine Club, National Organization devoted to honoring great dairy leaders of the past and present. With him are H. R. SEARLES (center) and J. P. EVES.

ALFRED M. GHORMLEY, president of Carnation Company, was named Guest of Honor for 1957 at the ninth annual meeting of the Dairy Shrine Club, national organization devoted to

# Why Filter Milk on the Farm?



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honoring great dairy leaders of the past and present, held at the Shrine's headquarters in Waterloo, Ia.

During ceremonies attended by breeders of purebred dairy cattle, dairy products manufacturers, educators and industry leaders, a portrait of Mr. Ghormley was unveiled. It will be hung in the Club's galleries.

Youngest of four sons of a Tacoma minister, Mr. Ghormley began his career in 1915 as a farm hand at Carnation Milk Farms, near Seattle. He soon was promoted to herdsman and later, when the farm manager resigned, he was given that responsible position in spite of his youth.

A natural judge of livestock, Mr. Ghormley also displayed keen administrative talents, technical ability, and sound judgment in developing the Carnation herd. His herd development program emphasized proper feeding and care of the Farms' animals in addition to bringing only those animals with the most desirable characteristics to the Farms for breeding purposes.

In 1929, shortly after Carnation entered the fresh milk and ice cream business, Mr. Ghormley was called from the farms to enter supervision in this field. In 1933, he became a Carnation vice president and director.

A year later he was appointed president of Albers Milling Company, Carnation's cereals and feeds subsidiary. In this capacity, Mr. Ghormley greatly influenced the development and improvement of the Albers line of farm feeds. In 1938, he returned to the fresh milk and ice cream division in full charge.

On February 22, 1957, Mr. Ghormley was elected president of Carnation Company after having held the important administrative post of assistant to the president since 1951.

## Univ. of Georgia News

F. W. BENNETT, who recently underwent a serious lung operation, will be recuperating for several weeks before resuming his duties at the University.

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The Univ. of Georgia took four out of five team first places in the Southern Dairy Products Judging Contest held in October at Athens, Ga. Teams participating were Georgia; Univ. of Maryland, College Park; Univ. of Kentucky, Lexington; North Carolina State tute, Auburn; Clemson College, Clemson, S.C.; West Virginia Univ., Morgantown, and Louisiana State Univ., Baton Rouge.

The first three team placings for the various divisions were as follows:

*All Products*—Georgia, Maryland, Kentucky; *Milk*—Georgia, North Carolina, Maryland; *Cheese*—Georgia, Maryland, Kentucky; *Butter*—Georgia, Kentucky, North Carolina, and *Ice Cream*—Alabama Polytechnic, Kentucky, Maryland.

Two Georgia milk and food sanitarians were given awards for outstanding service at the October meeting of the Georgia Chapter of the International Association of Milk and Food Sanitarians.

They are WILLIAM WEAVER, Columbus, who was given the award for the outstanding milk sanitarian for 1957, and EUCLE GEORGE, Perry, who received the award for the outstanding food sanitarian for 1957.

## Dairy Show Judging Contest Results

Ten collegiate judging teams competed in the regional dairy products judging contest held in October in connection with the International Dairy Show and sponsored cooperatively by the Show and the Chicago Society of Dairy Technology.

Teams participating in the event were Iowa State College, Ames; Univ. of Nebraska, Lincoln; Univ. of Missouri, Columbia; Ohio State Univ., Columbus; Univ. of Minnesota, St. Paul; Purdue Univ., West Lafayette, Ind.; Univ. of Illinois, Urbana; Univ. of Wisconsin, Madison; South Dakota State College, College Station, and Kansas State College, Manhattan.

Team placings in the various divisions were as follows:

*All Products*—Iowa, Illinois, Wisconsin; *Milk*—Iowa, Kansas, Wisconsin; *Butter*—Iowa, Illinois, Wisconsin; *Cheese*—Illinois, Iowa, Minnesota; *Ice Cream*—Missouri, Kansas, Iowa.

Individual first place winners in the various fields were:

*All Products*—JOHN FABRICIUS, Iowa; *Milk*—HAROLD SUELTER, Kansas; *Butter*—DON BAUMAN, Iowa; *Cheese*—RAY SPECKMAN, Illinois; *Ice Cream*—RICHARD FALLERT, Missouri.

Judges were B. J. ALDRICH, Chicago, Ill., *butter;* E. L. BYERS, Chicago, Ill., *milk;* W. J. CORBETT, Rockford, Ill., *ice cream;* H. SARTORIUS, Elmhurst, Ill., *cheese*. Superintendent of the contest was N. E. FACRICIUS, Ladysmith, Wis.

## Arkansas News

New additions to the staff of the Dept. of Animal Industry and Veterinary Science of the Univ. of Arkansas, Fayetteville, include H. W. CALVIN, JR., who will teach and conduct research in the field of dairy physiology. Dr. Calvin was formerly on the staff of the Dept. of Physiology, Oklahoma State Univ., Stillwater; PHILLIP VON ENGLER, who will assist in research work in the field of dairy manufacturing; and FRED E. REED, who will assist with research work in the field of dairy nutrition.

## Career Day

A very successful Dairy Manufacturing Career Day was held at the University of Connecticut, Storrs, in November. Dairy Industry

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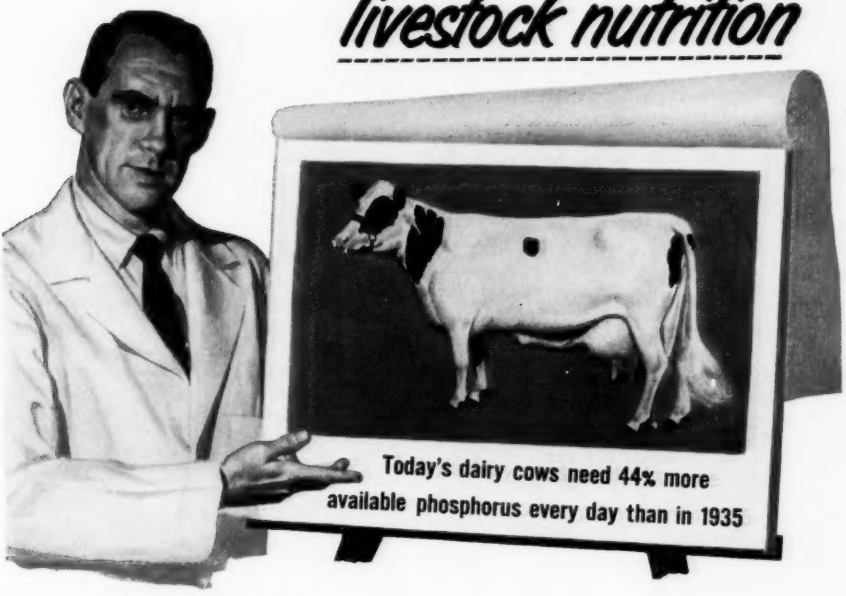


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personnel recruited the 75 students who attended from schools throughout the state.

Several short talks were given in the morning by dairy industry personnel and faculty on subjects dealing with costs of attending college, campus jobs, admission procedure, scholarships, armed-forces obligations, available curricula in both the two-year school and the four-year college program, and opportunities in the dairy industry after graduation.

The day included a tour of the dairy manufacturing facilities and campus, a luncheon handled by the Connecticut Milk Dealers' Association and the Connecticut Association of Dairy and Food Sanitarians, and attendance at the Connecticut–New Hampshire football game.

## A.P.I. Forms Alumni Group

At a recent breakfast meeting, alumni of Alabama Polytechnic Institute, Auburn, formed a Dairy Alumni Organization. Officers elected for the 1957-58 year are ROBERT STEVENSON, Opelika, *president;* PETE TURNHAM, Auburn, *vice-president;* C. J. JACOBS, West Point, *vice-president;* G. H. ROLLINS, Auburn, *secretary;* PARKER CHILDRESS, Opelika, *treasurer;* BILL POLIDORA, Shawmut, *director;* and ERNEST HUTTO, Pritchard, *director.*

W. H. EATON, recently retired from active teaching at A.P.I., was named honorary president of the organization.

## Western Div. Holds Judging Contest

The western division of the A.D.S.A. held its Intercollegiate Dairy Products Judging Contest during October at the Univ. of California, Davis. Eight universities and colleges entered judging teams.

The winners were California State Polytechnic College, San Luis Obispo, first; Oregon State College, Corvallis, second, and the Univ. of Idaho, Moscow, third.

## Meadow Gold Promotes D. L. Chew

D. L. CHEW has been promoted to assistant manager of the St. Joseph, Mo., Meadow Gold Dairy Plant. He joined Meadow Gold in 1954 as sales supervisor in the St. Joseph plant. Before that, he was owner-manager of the Harris Dairy Co., St. Joseph.

Mr. Chew is a graduate of Kansas State College, Manhattan, and served with the U. S. Air Force during World War II.

## Beatrice Promotes Sullivan

J. G. SULLIVAN has been promoted from assistant manager to manager of the Beatrice Foods Company dairy plant at Billings, Mont. He joined Beatrice Foods Company in 1951 as an office clerk at Great Falls, Mont., advanced to office manager in that plant, and then was appointed assistant manager at Billings.

A native of Butte, Mont., Sullivan served in the U. S. Navy for two years and is a business administration graduate of the College of Great Falls, Mont.

## Clemson News

JAMES C. SIMMONS has accepted a Dairy Industry Supply Association Fellowship. He will study at Clemson under the direction of D. M. GRAHAM.

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The Clemson Dairy Dept. is the recipient—for the sixth consecutive year—of a $2,500 grant from the Savannah Sugar Refining Corp. for research on the feeding of molasses to dairy cattle.

## Robinson to Cal. Polytechnic

C. R. ROBINSON, well-known dairy field man and former American Guernsey Cattle Club western regional field director, has been appointed to the Dairy Husbandry Dept., California State Polytechnic College, San Luis Obispo. He is substituting for RUSSELL NELSON, who is on a year's sabbatical leave to attend the Univ. of Missouri, Columbia.

## Montana News
## Annual Dairy Industry Week

The 22nd Annual Dairy Industry Week was held at Montana State College, Bozeman, Nov. 18 to 23. Topics discussed were: New Products for the Dairy Industry; Materials for Sanitation and Dairy Sanitation; Packaging Materials; Selling Dairy Products; Problems in Cottage Cheese Manufacture; Detecting Antibiotics in Milk; Farm Bulk Milk Tanks; Feeding Dairy Cattle; Disease Control; Efficient Production, and Clinics for Butter, Cottage Cheese, Ice Cream, and Milk.

Guest speakers were N. C. ANGEVINE, Meyer-Blanke Co., St. Louis, Mo.; H. BOWSER, Dairypak, Inc., Cleveland, O.; H. E. CALBERT, Univ. of Wisconsin, Madison; J. CATLIN, Veterinarian, Livingston, Mont.; M. W. CLARK, American Dairy Association, Chicago, Ill.; C. W. JENSEN, Michigan State Univ., East Lansing; F. KESSLER, Bozeman, Mont.; N. E. LAZARUS, Buffalo, N. Y.; J. MANDEVILLE, Bozeman, Mont.; E. PIRNER, Marathon Corporation, Menasha, Wis.; I. W. SLATER, Univ. of Wyoming, Laramie; L. H. MINOR, Wyandotte Chemical Corporation, Wyandotte, Mich., A. W. STORTI, Dept. of Physical Education, Montana State College; J. D. C. WIPF, Livestock Sanitary Board, Helena, Mont.

Dairy Industry Staff members were J. C. BOYD, J. L. BRENCE, R. R. HEDRICK, A. O. JACOBS, E. A. KEYES, J. A. NELSON, N. C. QUESENBERRY, and E. P. SMITH.

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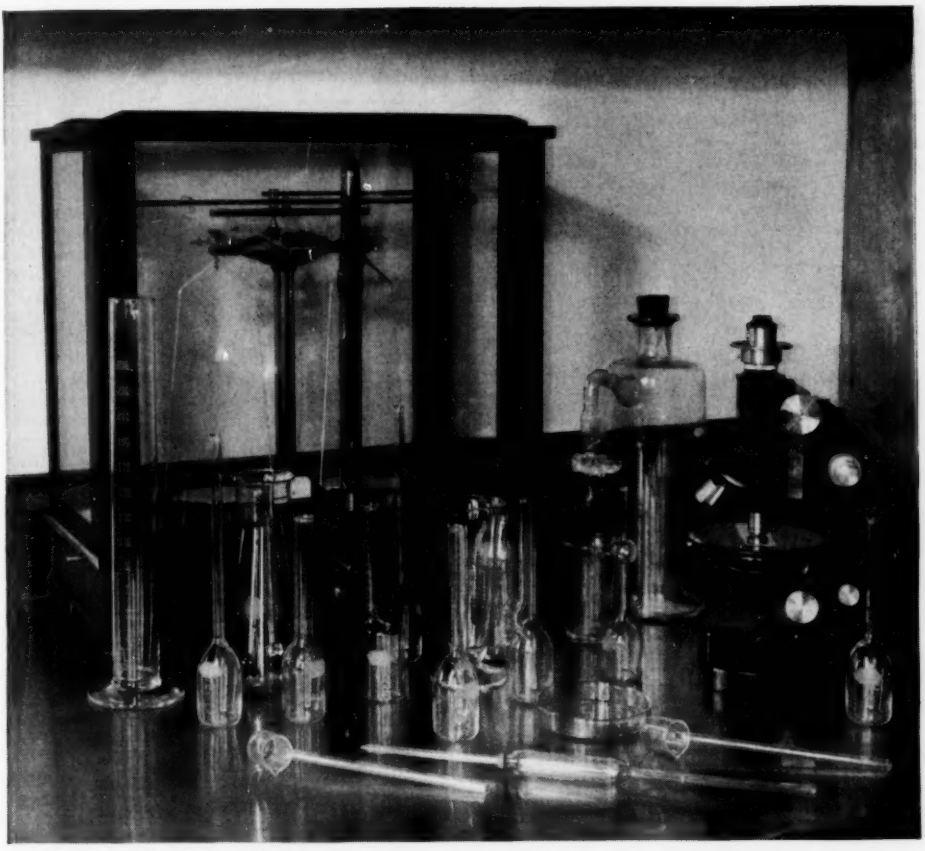
J. H. FRITZ, a 1941 graduate in dairy manufacturing from Montana State College, Bozeman, was recently appointed Chief of the Food



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and Public Health Inspection Division of the Bureau of Food and Public Health Engineering, USPHS, Washington, D. C.

Since his graduation from Montana State, he has served in the U. S. Army, and earned an M.S. degree in public health from the Univ. of Minnesota, St. Paul. In 1946 he joined the Food Section of the Kansas City Health Dept. where he advanced to the position of Chief, a post held until his recent appointment.

In 1956, Mr. Fritz was awarded the title "Sanitarian of the Year" by the International Association of Milk and Food Sanitarians for his accomplishments in the field of milk and food sanitation.

## Ritchie, Scaletti Join Minnesota Staff

J. RITCHIE, assistant professor in the Dept. of Dairy Science, Univ. of Manitoba, Canada, has joined the Dairy Dept. staff at the Univ. of Minnesota, St. Paul, as a Research Fellow.

J. V. SCALETTI, formerly with American Cyanamid Co., is now doing silage research on a U. S. Public Health Service grant in the Dairy Dept.

## Arizona Conference to Be Jan. 15-16

The Seventh Annual Dairy Husbandry Conference will be held at the Univ. of Arizona, Tucson, Jan. 15-16, 1958. Speakers will include H. C. FEDDERSEN, deputy director of the Dairy Division, Agricultural Marketing Service, USDA, Washington, D. C.; A. J. MORRIS, head of the Dairy Industry Dept., Utah State Univ., Logan, and G. D. ARMERDING, West Coast manager of the Mojonnier Bros. Co., Oakland, Calif.

## Meadow Gold Promotes Moody

HERBERT MOODY has been promoted to manager of Beatrice Foods Co.'s Meadow Gold Dairy in Kankakee, Ill.

Moody succeeds H. R. ALEXANDER who has been advanced to the post of manager of the company's Meadow Gold Dairy in Waukegan, Ill.

A native of Kankakee, Moody started with the Kankakee plant as a home delivery route salesman in 1941. Subsequently, he transferred to wholesale route salesman and advanced successively to sales supervisor and sales manager before becoming manager.

Alexander, also a native of Kankakee, started with the Kankakee milk plant in 1936 and has been manager since 1951.

## Jones Joins A.P.I. Staff

R. S. JONES, JR., Randolph County Agent, has become assistant dairyman for the Alabama Polytechnic Institute Extension Service. He succeeded OSCAR D. CROSBY, JR., who resigned to go into private industry.

A native of Barbour County, Jones received his B.S. degree in agriculture from Alabama Polytechnic Institute. He has served as Randolph County agent for the past nine years. Before that, he was assistant agent in the same county for seven years, with a short time out for military duty.

## Penn State News

The top-ranking students in the short-term ice cream manufacturing courses at Pennsylvania State Univ. and the Univ. of Maryland, College Park, will receive awards from the American Food Laboratories. The firm develops and manufactures flavor ingredients for ice cream products and other foods.

An inscribed gold wrist watch will be presented by the company to the student with the highest grades at the completion of the two weeks' course in ice cream manufacture given by the Colleges of Agriculture at the end of the 1958 short courses. The company plans to expand the awards project further in 1958, by making similar awards at Ohio State Univ., Columbus, and Michigan State Univ., East Lansing.

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P. S. WILLIAMS of the diary husbandry staff has resumed his duties in the Dairy Science Dept. after a sabbatical leave during which he and Mrs. Williams traveled about 16,000 miles via car and trailer. They toured through 30 states and part of Mexico, visiting 27 colleges and experiment stations and renewing friendships with many former students, relatives, and acquaintances.

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Some 40 staff members and 60 undergraduate students and their families attended a chicken barbecue sponsored recently by the Penn State Dairy Science Club.

## NDC Annual Meeting Is Jan. 27-29

"Education: The Priceless Ingredient," will be the theme for the 43rd Winter Conference–Annual Meeting of the National Dairy Council to be held at the Dayton-Biltmore Hotel, Dayton, O., Jan. 27-29.

Subjects to be covered will include: The Diet–Heart Disease Problem; What Science Says About Dairy Products; Motivating People to Use Dairy Products; How Dairy Council Contributes to the Sale of Dairy Products; Dairy Industry Responsibility in Dairy Council Management.

Prominent participants in the event will include: P. L. WHITE, secretary of the Council on Foods and Nutrition of the American Medical Association; W. F. ALEXANDER, Pastor, The First Christian Church, Oklahoma City; and S. A. ROBERT, Market Research Director, American Dairy Association. The following NDC Board members will also participate: W. A. FOSTER, The Borden Cheese Co.; W. E. WINN, Pure Milk Association, Chicago; P. P. MILLER, National Dairy Products Corp., New York City; MOSE KISER, Builford Dairy Co-

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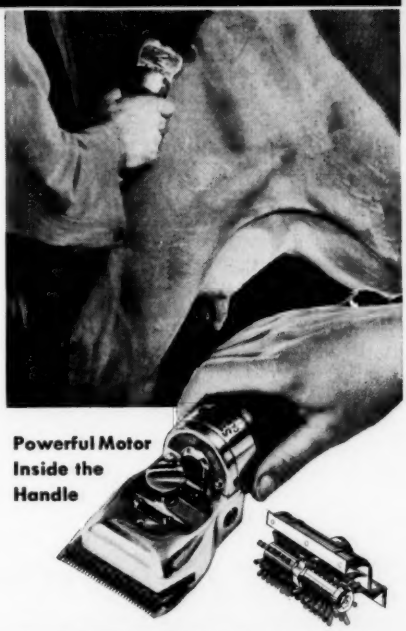


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op Association, Greensboro, N. C.; L. C. Mapp, Miami Valley Milk Producers Association, Dayton, Ohio; G. S. Bulkley, Carnation Company, Los Angeles (Chairman of the NDC Board of Directors), and Milton Hult, NDC President.

### M.S.U. Dairy Engineering Meeting

The Sixth Annual National Dairy Engineering Conference will be held Feb. 25-26, 1958, in Kellogg Center, Michigan State Univ., East Lansing. The event is sponsored by the M.S.U. Depts. of Agricultural Engineering and Dairy.

### Recent Deaths

James Tallman Smith, 48, research engineer for The Creamery Package Manufacturing Company, Chicago, died suddenly of a heart attack on Oct. 10, 1957, at Ames, Ia.

A graduate of Ohio State Univ., Columbus, Mr. Smith was widely known in the dairy industry. He had served many years as a milk sanitarian for the city of Steubenville, O., Health Dept.; production superintendent of the Telling-Belle-Vernon Milk Company, Cleveland, O., and extension specialist for the Dept. of Dairy Technology at O.S.U. For the past three years he worked in the Research Engineering Dept. of The Creamery Package Company.

A resident of Whitewater, Wis., he is survived by his widow, Beulah; a daughter, Martha Jean, and a son, James Loring. Burial was at Raymond, O.

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George P. Sanders, 62, a chemist in the Dairy Products Section of the Agricultural Research Service, USDA, died Oct. 12, after undergoing a major surgical operation.

Dr. Sanders received his B.S. and M.S. degrees from the Univ. of Minnesota, St. Paul, and his Ph.D. degree from the American Univ., Washington, D. C. After serving as an army officer in World War I, he served as a county agent for the Minnesota Agricultural Extension Service, 1919-1920; high-school instructor, 1920-22, and employee of the U. S. Veterans Administration, 1923-25.

In 1926, he joined the Bureau of Dairy Industry of the USDA (this unit is now called the Dairy Products Section of the Agricultural Research Service), where he served until his death.

The USDA presented him with the Superior Service Award in 1950 for his development of a phosphatase test for the purity of pasteurized products. Another of Dr. Sanders' achievements was a method for dehydrating cheese, which was credited during World War II with a significant saving of shipping space in transporting food supplies overseas.


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Dr. Sanders published more than 50 technical papers in his field. His subjects included the physical and chemical properties of milk, minerals in milk, fractionation of milk protein, chemistry of cheese, bacterial fermentations, and quality improvement in dairy products.

His handbook, *Varieties and Descriptions of Cheese*, has been called a classic in its field. He was co-author of two chapters and associate editor of a nearly completed reference text, *The Fundamentals of Dairy Chemistry*. He is survived by his widow, Blanche; a daughter, Mrs. Stanley Atma, Cleveland, O., and a son, George P. Sanders, Jr., Arlington, Va. Burial was in Arlington Cemetery.

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ROBERT J. REMALY, 47, an executive with the American Dry Milk Institute, Chicago, died suddenly Nov. 15 of a heart attack.

Mr. Remaley, a widely known figure in the dairy industry, had been on the Institute staff in charge of scientific development and of the Laboratory Dept. since 1951. During World War II he was head of the Dairy Branch of the Quartermaster Corps Subsistence Researc and Development Laboratory in Chicago. Before the war, he was with Kraft Foods Company, Chicago.

He is survived by his widow, Gail, and a daughter, Harriet. Burial was in Ridgewood Cemetery, Des Plaines, Ill.

## AAAS Annual Meeting Is December 28-30

The annual meeting of the American Association for the Advancement of Science will be held in Indianapolis, Ind., Dec. 28-30, 1957. Section O of the A.A.A.S. (of which the A.D.S.A. is an affiliated society) has planned a symposium on "Chemical and Biological Control of Plant and Animal Pests."

All meetings will be held in the Bamboo Room of the Washington Hotel. A résumé of the program follows:

*Morning, Dec. 28*—"Insecticides," J. C. KELLER, entomologist, Agricultural Research Service, USDA; "Herbicides," R. H. BEATTY, director, Agricultural Chemicals Division, American Paint Co.; "Fungicides and Bactericides for Control of Plant Diseases," G. L. MCNEW, managing director, Boyce Thompson Institute for Plant Research, Inc.; "Chemical Control of Internal Parasites of Domestic Animals," F. O. GOSSETT, veterinarian, The Lilly Research Laboratories; "Systemic Antibiotics and Chemicals: Their Movement and Mode of Action," J. E. CASIDA, assistant professor of entomology, Univ. of Wisconsin.

*Afternoon, Dec. 28*—"Parasites and Predators for Pest Control," C. A. FLESCHNER, associate entomologist, Univ. of California; "Control of Forest Insects," J. A. BEAL, entomologist, USDA; "Control of Forest Diseases," J. R. HANSBROUGH, plant pathologist, USDA; "Pathogens for the Control of Pests," J. D. BRIGGS, associate entomologist, Illinois State Natural History Survey; "Antagonism as a Plant Disease Control Principle," W. C. SNYDER, plant pathologist, Univ. of California; "Irrigation for Pest Control," E. F. KNIPLING, director, Entomology Research Division, Agricultural Research Service, USDA.

*Afternoon, Dec. 29*—"Disease Resistance in Animals: Some Genetic Implications of the Avian Leukosis Complex," N. F. WATERS, poultry geneticist, Agricultural Research Service, USDA; "Breeding Plants for Resistance to Insect Pests," R. H. PAINTER, professor of entomology, Kansas State College; "Breeding Vegetable and Fruit Crops for Resistance to Diseases," J. R. SHAY, head, Dept. of Botany and Plant Pathology, Purdue Univ.; "Breeding Field Crops for Resistance to Diseases," E. H. STANFORD, associate professor of agronomy, Univ. of California; "Nutrition of the Host and Reaction to Pests," J. G. RODRIGUEZ, associate entomologist, Univ. of Kentucky.

*Morning, Dec. 30*—"Biological Balance as Affected by Disease and Insect Control Practices," A. D. PICKETT, officer in charge, Crop Insect Section, Science Service Laboratory, Canadian Dept. of Agriculture; "Exclusion and Eradication versus Reduction in Diseases and Pests," M. R. CLARKSON, deputy administrator, Agricultural Research Service, USDA; "Educa-

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tion in the Use of Pesticides," E. H. FISHER, associate professor of entomology, Univ. of Wisconsin; "Effects of Regulatory Control on Evaluations of Safety and Suitability," B. L. OSER, director, Food Research Laboratories, Inc.

## Kentucky News

After 27 years on the staff of the Dairy Section of the Univ. of Kentucky, Lexington, H. B. MORRISON has resigned in order to continue his work with the International Cooperation Administration of the U. S. Dept. of State. For the past two years he has been on leave from the University so that he could serve the I.C.A. in Cochabamba, Bolivia.

Dr. Morrison received his B.S. and M.S. degrees from the Univ. of Minnesota, St. Paul, and his Ph.D. degree from Iowa State College, Ames. As a staff member at Kentucky he taught and did research in dairy bacteriology.

In 1955 he joined the staff of the I.C.A. to supervise the construction of a dairy processing plant in Cochabamba, Bolivia. His present assignment with the I.C.A. is to return to Bolivia to complete the work started during his first tour of duty in that country.

A member of several social and professional organizations, Dr. Morrison in 1954 was named honored guest by the Univ. of Kentucky Dairy Club. This is the highest award that can be given a faculty member or industry leader by the undergraduates of the Dairy Section, Univ. of Kentucky.

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G. E. WILLIAMSON has been named Inspector-in-Charge of the Creamery License Section of the Univ. of Kentucky. He succeeds J. D. FOSTER, who has held that position for the past 33 years.

Mr. Williamson, a graduate of the Univ. of Missouri, Columbia, served as a county agent in Graves County, Ky., from 1935 to 1948, except for four years when he served in the U. S. Army during World War II. He was made a field agent in dairying at the University in 1948, a position he held until his recall to active Army service in 1951. In the fall of 1957, he left active duty in the Army with the rank of Lieutenant Colonel.

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J. L. BUCY, a graduate of Murray State College, has been named field agent in cream grading for the Dairy Section.

A veteran of World War II, Mr. Bucy served as an instructor for the Veterans Administration training program in Grayson County, Ky. He was later appointed district field man for the Sugar Creek Creamery Co., a position he held until his recent appointment at the University.

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More than 50 students in dairying were guests of the faculty of the Dairy Section at a dinner held recently at Lexington.

The Student-Faculty Mixer is an annual event sponsored by the faculty so that teachers may become better acquainted with their students. D. M. SEATH, head of the Dairy Section, presided over the program.

RICHIE LOWE, president of the Kentucky Dairy Club, spoke about plans for the Dairy Club during the coming year. Besides service functions at various cattle sales and short courses, the Club will publish an Annual, Sponsor the Homecoming Breakfast, and conduct an Honor Banquet in connection with Dairy Career Day.

## Completed Theses

*M.S. Degree*

MAURICE COLE—How supplementing lactating dairy cows on summer pasture with either alfalfa hay or alfalfa silage affected their dry matter intake. Univ. of Kentucky, Lexington.

H. J. COOK—Soilage feeding for dairy cattle. Univ. of Rhode Island, Kingston.

C. J. COSGROVE—Evaluation of methods for determining total solids, butterfat, and solid-not-fat content in milk. Univ. of Rhode Island, Kingston.

CARL L. DAVIS—The value of urea and dicyandiamide for lactating dairy cows. Univ. of Kentucky, Lexington.

DONALD R. DOWDEN—A comparison of zero, low, and high level grain feeding to lactating cows on permanent, improved, and supplemental pastures. Univ. of Kentucky, Lexington.

JAMES P. EVERETT, JR.—The effect of protein levels of starters with and without aureomycin on the growth rate and metabolism of young dairy calves. Univ. of Kentucky, Lexington.

ROBERT W. WELK—Melting properties of Cheddar cheese. Univ. of Minnesota, St. Paul.

G. W. WILDES—The value of grass-legume silage as a sole roughage for dairy cattle. Univ. of Rhode Island, Kingston.

*Ph.D. Degree*

N. S. FECHHEIMER—Chromatin variations in bovine spermatogenic cells. Ohio State Univ., Columbus.

AHMED A. HASSAN—Input, output relationship and milk production. Univ. of Minnesota, St. Paul.

LLOYD R. HUNSAKER—The comparative influence of sires used in artificial insemination vs. those used naturally on butterfat production. Univ. of Minnesota, St. Paul.

## Young Returns to Oregon

J. O. YOUNG, assistant dairy technologist, has returned to Oregon State College, Corvallis, after spending a year's sabbatical leave studying at the State College of Washington, Pullman.

## News from Ohio State

Forty students, representing 30 different companies, attended the first session of the new Market Milk Correspondence Course held recently at Ohio State Univ., Columbus.

The outstanding feature of the course is that the assignments of lessons are to be completed at home. This feature makes it possible for more men from smaller companies to participate. The course consists of 18 home lessons and five contacts of one day each at the Dept. of Technology.

The picture below shows part of the correspondence course class receiving instructions in dairy bacteriology. At the microscope is T. V. ARMSTRONG; standing at the table is C. L. HANKINSON.

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T. Scott Sutton, associate dean of the College of Agriculture, has returned to the O.S.U. campus from India, where he served as administrator of the Ohio State Univ. agricultural education program. The seven-member team from O.S.U., in cooperation with the Indian government, established four agricultural and veterinary colleges.

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High lights of the November meetings of the Ohio dairy technology societies were clinics and discussions on Cultured Buttermilk and Salad Cream. W. L. Slatter, Dept. of Dairy Technology, conducted the clinics and led the discussions. Societies participating were Cincinnati, Central Ohio, Northeastern Ohio, and Maumee Valley.

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E. F. Almy, professor of agricultural biochemistry, was honored by the Dept. of Dairy Technology at the Ninth Annual Homecoming Brunch. Approximately 150 friends and former students of Dr. Almy attended.

As a member of the agricultural biochemistry staff, Dr. Almy for many years has been teaching dairy chemistry to dairy technology majors. In appreciation for his excellent cooperation over the years, he was presented with a Certificate of Appreciation by the Dept. of Dairy Technology. The presentation was made by I. A. Gould, chairman of the Department.

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About 65 students and staff members attended the Annual Bean Feed held recently in honor of new freshmen in the Dept. of Dairy Technology. The event is sponsored jointly by dairy technology upperclassmen and staff members of the department.

As part of the program, the functions of the O.S.U. Dairy Club and the A.D.S.A. were explained to the new students by various officers of the club. The evening concluded with an official welcoming address by Dr. Gould.

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The Silver Jubilee Meeting of the Ohio State Dairy Technology Conferences will be held Feb. 25-28, 1958, at Columbus, under the sponsorship of the Dept. of Dairy Technology.

Visiting staff members on the program include W. B. Combs, Univ. of Minnesota, St. Paul; I. Parkin, Pennsylvania State Univ., University Park; H. C. Olson, Oklahoma State Univ., Stillwater, and E. W. Bird, Iowa State College, Ames.

On this year's program, Butter Day has been changed to Manufactured Dairy Products Day. Furthermore, consideration is being given to dividing the main sessions into smaller groups, to encourage more individual participation.

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Philip L. Haymes, director of United Milk Products Company, Cleveland, O., was presented the *Award of Merit* at the Ninth Annual Dairy Technology Homecoming Brunch. The award, which has been presented annually since

1949 by the Dept. of Dairy Technology, is for outstanding contributions to the Dairy Industry. The presentation was made by L. L. RUMMELL, dean of the College of Agriculture.

Following high school, Mr. Haymes went to work for Western Reserve Condensed Milk Company in 1906 in the Kent, O., plant. Since then he has held every operating and executive position in Western Reserve, which later became United Milk Products Company. He now serves as a director of the company.

Mr. Haymes early recognized the importance of science in the dairy industry and managed to wedge in six years of college training, practically all science, while working. He is a member of the American Chemical Society and a life trustee of the Cleveland Branch of the Y.M.C.A.

His first contribution, nationwide, was his work on the United States Food and Drug Adminitration's original milk bread standards in 1923. Since then, he has served on several Food and Drug Administration committees concerning standards for milk, and was also instrumental in getting standards for evaporated, condensed, and concentrated milk in the Ohio General Code.

Throughout World War II, he served on the War Food Administration's committee, representing the evaporated milk industry. He also served on several other wartime and regulatory committees and in 1952 was chosen to represent the dairy industry before the United States Senate and House committees on termination of controls under the Defense Act.

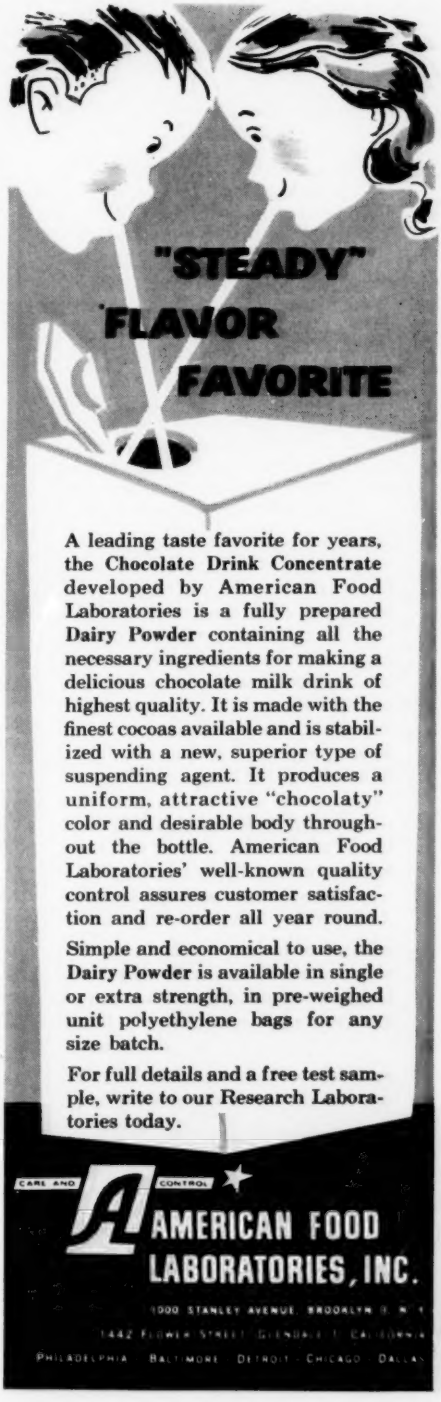
Mr. Haymes is a member of the 13-man advisory group to Secretary of Agriculture, E. T. BENSON. For years he has been a member of USDA dairy advisory committee on research and marketing, serving as chairman from 1955-1956.

## News from Borden's

JACK B. PENTZ, executive vice-president of Borden's Dairy and Ice Cream Company, Columbus, O., has been named chairman of Borden's Midwest fluid milk and ice cream district. The district operates fluid milk and ice cream plants in nine Midwestern states.

Mr. Pentz succeeds NORVAL D. GOSS, who retired as president of Borden's Dairy and Ice Cream Company and as district chairman. Mr. Goss, however, will continue as chairman of the board of the Columbus company and of Borden's Michigan Milk and Ice Cream Division. Mr. Goss joined Borden's in 1915 as an accountant and rose through the ranks to become chairman of the Midwest district in 1939.

Pentz joined Borden's Moores and Ross of Columbus in 1928 as supervisor of its product control laboratory. By 1946 he became manager of Moores and Ross; and three years later he was promoted to vice-president of the Dairy and Ice Cream Company and director of sales and advertising for the Midwest district. He was appointed executive vice-president in 1956.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# STUDENT CHAPTER NEWS

A section devoted to the activities of dairy students

E. L. THOMAS, *Editor*

## The Objectives of a Local Dairy Club Program

JAMES E. JOHNSTON

*Associate Professor of Dairying, Louisiana State University and Agricultural and Mechanical College, Baton Rouge, La.*

Two years ago the Student Affiliate Committee of the A.D.S.A. sent, to each affiliate chapter, a list of suggested objectives. These were as follows:

1. Develop closer relationships among students through strong local club programs.
2. Develolp closer relationships among students, faculty, and industry and among educational institutions.
3. Acquaint students with the American Dairy Science Association, its scope, purpose, and the role of affiliate members in the over-all program.

I should like to add two more:

4. Develop and maintain interest among students and prospective students in dairying as a career.
5. Provide an opportunity for recreational activities.

The success or failure of a dairy club depends, to a large degree, upon how well leadership development is carried on. This has been cited as the number one objective above and certainly must be accomplished before any of the other objectives can be attained. While some individuals make better leaders than others, leadership is a trait which can be developed in nearly any college student by proper training. This can best be accomplished in the dairy club by placing all new members on committees and gradually increasing their responsibilities as they gain experience. Such a program develops students who are qualified to fill the key offices of the club as upperclassmen and who enter the dairy industry with the ability to work with others as graduates.

Another critical factor in the long time success of the dairy club is the attitude of the faculty advisor. The advisor who shoulders the responsibility of running the club himself is certainly doing an injustice to his students. However, he must bear the responsibility of seeing that the club objectives are carried out. He can best do this by keeping in close touch with club officers to insure that they do not shirk their responsibilities. Without close faculty supervision a club tends to "run down" and

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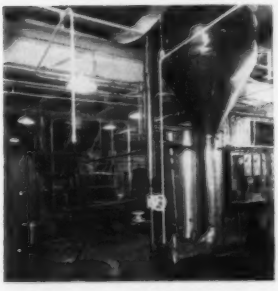

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gradually lose sight of its objectives. The advisor must be an individual who is interested in students and their problems and who is willing to devote a great deal of his own time to the job.

The objectives of the club having been established, a plan of programs and activities must be set up to accomplish them. Such a plan should be outlined at the beginning of each school year. First consideration should be given to programs on subjects not usually available to the students from some other source. The topics reported in the 1956 "Annual Reports of Affiliate Club Activities" include many which should have been informative and worthwhile; however, some, such as "Talk on Mastitis" or "The Use of Sodium Metabisulfite as a Preservative for Silage," might have been better reserved for class discussion. Talks by campus visitors representing farm groups, industry, or foreign countries give the membership an opportunity to broaden their outlook in a manner not otherwise possible. Each club activity should be judged on the basis of its contribution to the student or the dairy industry. Some purely social activities are excellent means of maintaining student interest and providing a break in the routine of campus life; however, this aspect can easily be overemphasized. Our busy campuses already provide too many activities for us to condone the existence of a club which does not have a well planned program designed to carry out worthwhile objectives.

The scrapbook exhibits prepared for the A.D.S.A. Annual Meetings during the past two years indicate that our clubs are generally doing a good job. In most cases, however, further improvement is possible through the development of programs planned to accomplish the objectives of the clubs. The Student Affiliate meetings held during the National A.D.S.A. meetings give student representatives and their advisors an opportunity to discuss their programs and problems. Through these contacts we should be able to continue to improve our student chapters. It is to be hoped that more student representatives will be able to attend these meetings in the future.

## California State Polytechnic College Dairy Cattle Judging Team Competes at Waterloo

The dairy cattle judging team of the California State Polytechnic College placed third in the all-breeds division of the national contest held at Waterloo, Iowa. The team placed second in Brown Swiss, fourth in Guernseys, fifth in Jerseys, and seventh in Holsteins. It was coached by DR. ALBRIGHT.

MRS. MARGUERITE S. TYSON of James Canyon Ranch, Genoa, Nevada, donated a Guernsey heifer to the judging team in order to help out with its traveling expenses. The heifer was sold for $360.00 at the California State Guernsey Sale.

## Virginia Tech Chapter News

FRED SCOTT, President of the Virginia Tech Chapter of A.D.S.A., has received another high honor. His name will appear in the next issue of "Who's Who Among College Students." Fred is a senior in the Department of Dairy Science, and is enrolled in the Dairy Technology option. He has been a member of the Dairy Products Judging Team and was assistant editor of the 1956 dairy annual, as well as participating in numerous other dairy club activities. In other extracurricula activities, Fred was a member of the Civilian Student Senate in 1956-1957 and also Treasurer of the Civilian Student Body in the same year. All fellow members of the Dairy Club are proud of Fred's numerous accomplishments.

Members of the club have had a very busy fall quarter. Among the activities carried out, the most important ones were entering a float in the Homecoming Parade, having an exhibit in the Horticulture show, and sponsoring the Dairy Cattle and Dairy Products Judging Teams in intercollegiate competition. Club members are now planning a gift cheese sales campaign, a Dairy Science Career Day for prospective dairy students, and another dairy annual. Hard work and cooperation among all is the theme of all members in carrying out these objectives.


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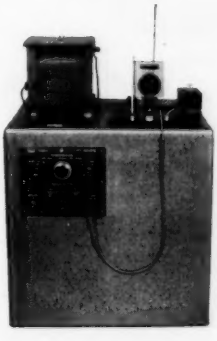

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It is with deep pride that we, the members of the dairy club, announce that PROFESSOR PAUL M. REAVES, faculty advisor for 1956-1957 received the Wine Teaching Award for 1957 in the School of Agriculture. Professor Reaves has made an outstanding contribution to the dairy industry, and we are all proud of this honor he so greatly deserves.

## From the Secretary's Desk

J. B. FRYE, JR., of the Univ. of Louisiana, has done an excellent job of organizing the membership campaign for 1958. Suggestions and aids have been sent to every state chairman and his co-workers.

Dues notices were sent to 1957 members on Oct. 15 and they have been coming in very well. As of Nov. 4, approximately 700 have paid dues for 1958 and of that number about 35 are new members.

The total membership for 1957 was 2,250. This year we hope to get 2,400. The number of new members necessary to reach this goal will depend on the number of 1957 renewals. We should do better than last year, when 200 of the 1956 members failed to renew for 1957.

Last year we had 606 Affiliate members. This year to date 106 have paid dues for 1958 and 27 of them are new.

If every member will consider himself a part of the Membership Committee, it will be possible to contact prospects and insure our goal of 2,400 new members.—H. F. JUDKINS.

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# NITROGEN FACTOR FOR THE CALCIUM CASEINATE–CALCIUM PHOSPHATE COMPLEX IN MILK

T. F. FORD, G. A. RAMSDELL, SHIRLEY G. LANDSMAN,[1] AND T. G. ALEXANDER[2]

*Eastern Utilization Research and Development Division, Agricultural Research Service, USDA, Washington, D. C.*

**The casein colloid in milk contains a phosphoprotein which has the same phosphorus : nitrogen ratio for all the broad intermediate ranges of particle sizes. The purpose of this work was to determine the nitrogen factor. For many milks and many particle-size fractions, the average ratio of dry weight of calcium caseinate–calcium phosphate complex was about 6.89 ± 1.3%, and this applies to normal milk. This factor does not apply to the largest casein colloid particles and may not apply to the smallest.** *Editor.*

To calculate densities, hydration, voluminosity, and other constants of the casein-containing particles in milk, a factor for conversion of grams of casein nitrogen to grams of dry casein complex is necessary. Experiments previously reported *(3)* indicate that the casein colloid in milk, in the broad intermediate range of sizes, contains a phosphoprotein which has the same phosphorus : nitrogen ratio in all the sizes of particles. This phosphoprotein is apparently present as a calcium salt, which is closely associated with excess calcium and phosphorus in the proportions in which these elements exist in tricalcium phosphate. The combination is frequently referred to as a complex. A definite minimum amount of such calcium phosphate seems to be always present *(3)*, and an excess amount which differs as between milks and over the particle-size range. The differences in over-all composition must be taken into account in assigning a nitrogen factor. This is here done by relating the factor to the calcium content. The factor calculated does not refer to the total micelle or particle, but only to the dry calcium caseinate–calcium prosphate complex contained in it; thus excluding the salts, lactose, and serum proteins which are also present.

## EXPERIMENTAL PROCEDURE

Skimmilk samples were centrifugally fractionated, using techniques previously described *(3)*. Liquid samples progressively depleted of the casein colloids, and deposited fractions of these colloids were obtained. The deposits were washed in most cases, by redispersing them in distilled water and centrifuging.

Both gravity and centrifugally separated skimmilks were used. The milks were not treated in any way and no preservatives were added. All samples were stored at 4° C. The methods of analysis are given in the previous publication *(3)*.

Received for publication March 3, 1957.

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[2] Present address, Food and Drug Administration, Washington, D. C.

FACTORS BASED ON ANALYSES OF LIQUID SAMPLES

Figure 1 is a typical plot showing the relationship between per cent total solids and per cent total nitrogen in skimmilk samples, as the casein-containing colloids are progressively removed by centrifuging. The serum nitrogen value shown was obtained by extrapolating the accompanying casein nitrogen : total nitrogen plot to zero casein nitrogen. This particular experiment was done in April, 1955. It has been previously reported *(3)*, but without the total solids data.

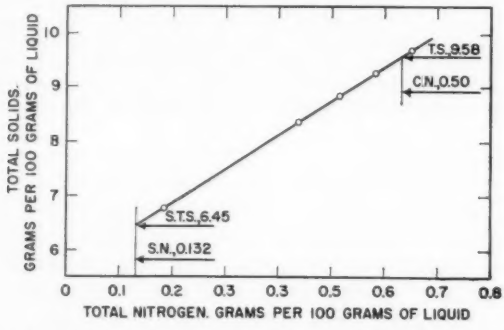


FIG. 1. Relationship of total solids to total nitrogen for skimmilk samples centrifuged for different lengths of time. *S.T.S.* = serum total solids. *S.N.* = serum nitrogen. *C.N.* = casein nitrogen. Ca = calcium. For this particular skimmilk, the ratio of casein nitrogen removed to total nitrogen removed is 1.00; the ratio of calcium removed to casein nitrogen removed is 0.181.

The method of calculating the complex : casein nitrogen factor, or ratio, from plots such as Figure 1, is similar to that used for calculation of the calcium : casein nitrogen ratio, as previously described *(3)*.

It is convenient to base the calculations on the analysis of the casein-free serum, as given by extrapolation, and on the analysis of an arbitrary skimmilk containing, for convenience, 0.5% casein nitrogen. A first approximation is given by the following equation: (1)

$$\text{Complex}/C.N. = \left[\frac{T.S. - 0.9476\ S.T.S. - (N.C.N. - 0.9476\ S.N.)\,(6.38)}{C.N.}\right]\left[0.995\right].$$

Here *C.N.* means casein nitrogen; *T.S.* means total solids, at 0.5% *C.N.*; *S.T.S.* means (centrifugal) serum total solids, at zero casein nitrogen; *S.N.* means (centrifugal) serum nitrogen, at zero casein nitrogen, and *N.C.N.* means noncasein nitrogen (total nitrogen less casein nitrogen), at 0.5% *C.N.*, a value usually identical or nearly identical with the centrifugal serum nitrogen. These values are taken from the plots at zero and 0.5% *C.N.* The numerical constant, 0.9476, represents the weight of casein-free serum in 1 g. of skimmilk, at 0.5% *C.N.*, assuming a complex : casein nitrogen factor of 6.89 and a hydration of

0.52 g. of hydrate water per g. of dry colloid.[3] The constant, 6.38, is the factor for conversion of serum nitrogen to serum protein. The multiplier, 0.995, is a correction factor for the fats, phospholipids, and other ether-extractable substances removed with the colloid by centrifugation.

Substituting the first approximate complex : nitrogen factor given by Equation 1 for 6.89, and recalculating, gives in every case a second approximation which differs negligibly from the first.

Complex : casein nitrogen factors calculated as indicated for several milks, including the one for which the data are shown (Figure 1), are listed (Table 1)

TABLE 1

*Relationship of the complex : casein nitrogen factor to the calcium : casein nitrogen ratio, based on analysis of progressively depleted samples of skimmilks; together with the serum nitrogen and the total solids values used in the calculations*

| Date | *S.N.*[a] | *S.T.S.* | *TS* at 0.50% *C.N.* | Ca/*C.N.* | Complex /*C.N.* | Milk |
|---|---|---|---|---|---|---|
| | *g/100 g.* | *g/100 g.* | | *g/g* | *g/g* | |
| October, 1946 | 0.165 | 6.86 | 9.99 | 0.182 | 6.83 | Jersey |
| December, 1948 | 0.134 | 6.68 | 9.84 | 0.207 | 6.90 | Herd |
| April, 1951 | 0.132 | 6.45 | 9.58 | 0.181 | 6.81 | Jersey |
| August, 1951 | 0.127 | 7.06 | 10.26 | 0.198 | 7.02 | Jersey |
| June, 1954 | 0.139 | 7.10 | 10.24 | —— | 6.91[b] | Jersey |
| March, 1955 | 0.119 | 6.75 | 9.84 | —— | 6.78[b] | Jersey |
| August, 1955 | 0.119 | 6.29 | 9.40 | 0.182 | 6.77 | Jersey[c] |
| August, 1955 | 0.140 | 6.26 | 9.45 | 0.184 | 6.90 | Holstein[c] |
| Averages | 0.134 | 6.68 | 9.83 | 0.189 | 6.872[d] | |

[a] *S.N.* = serum nitrogen; *S.T.S.* = serum total solids; *C.N.* = casein nitrogen.
[b] Omitted in averaging, because calcium was not determined.
[c] About 20% of the colloid was removed from these two skimmilks by preliminary centrifugation. These results, therefore, apply to intermediate and small particle sizes only.
[d] Standard deviation, 0.081.

together with the serum nitrogen and total solids values on which the calculations were based, and together with the calcium : nitrogen ratios for the complex, where these were determined. Since the total solids : total nitrogen plots are in every case straight lines within the accuracy of the data, the factors given are averages for the ranges of particle sizes removed. The first four experiments, which include the April, 1951, experiment, have all been reported previously, in part *(3)*.

## FACTORS BASED ON ANALYSES OF DEPOSITS

Complex : casein nitrogen factors and calcium : casein nitrogen ratios, based on analyses of several centrifugally deposited casein colloids, are given (Table 2). The original data for the first two sets of values are given in the paper referred to previously.

[3] 100-(0.5)(6.89)(1.52) = 94.76. The hydration, 0.52 g. per g. of dry complex, is based on results by deKadt and van Minnen *(1)*, who, however, refer their values to casein. Their average hydration, 0.55 g. per g., multiplied by the ratio of the casein : nitrogen factor, 6.45, to the assumed complex : nitrogen factor, 6.89, is 0.52 g. per g. DeKadt and van Minnen's values vary quite widely, but we have repeated their experiments and obtained about the same results. The effect of variations in hydration will be considered in the discussion.

TABLE 2

*Relationship of the complex:casein nitrogen factor to the calcium:casein nitrogen ratio, for various centrifugally deposited casein colloids*

| Ca/*C.N.*[a] | Complex/*C.N.* | Milk | Particle-size fraction | Approximate per cent of total complex |
|---|---|---|---|---|
| | | Colloids not washed | | |
| 0.216 | 7.18 | Jersey 1 | Largest | 2 |
| 0.211 | 7.01 | Jersey 1 | Large | 3 |
| 0.200 | 7.06 | Jersey 1 | Intermediate | 3 |
| 0.198 | 7.03 | Jersey 1 | Intermediate | 3 |
| 0.191 | 7.02 | Jersey 1 | Intermediate and small | 70 |
| 0.207 | 6.92 | Jersey 2 | Composite | 80 |
| 0.204 av. | 7.04 av. | | | |
| | | Colloids washed once with distilled water | | |
| 0.205 | 6.95 | Jersey 3 | Large | 7 |
| 0.217 | 7.12 | Jersey 3 | Intermediate | 10 |
| 0.203 | 7.02 | Jersey 3 | Intermediate | 20 |
| —— | 6.92 | Jersey 3 | Intermediate and small | 50 |
| 0.200 | 6.90 | Jersey 4 | Composite | 80 |
| 0.206 av. | 7.00 av. | | | |
| | | Colloids washed twice with distilled water | | |
| 0.190 | 6.81 | Herd 1 | Intermediate | 60 |
| 0.207 | 6.91 | Herd 2 | Intermediate | 60 |
| 0.190 | 6.75 | Jersey 5 | Intermediate | 60 |
| 0.194 | 6.94 | Jersey 6 | Composite | 80 |
| 0.200 | 6.80 | Jersey 7 | Composite | 80 |
| 0.209 | 6.85 | Jersey 8—24 hr.[b] | Composite | 80 |
| 0.215 | 6.91 | Jersey 8—48 hr.[b] | Composite | 80 |
| 0.202 | 6.83 | Jersey 8—1 wk.[b] | Composite | 80 |
| 0.204 | 6.96 | Jersey 9 | Intermediate | 60 |
| 0.206 | 6.87 | Jersey 9 | Composite | 80 |
| 0.232 | 6.95 | Jersey 9 | Large | 10 |
| 0.204 av. | 6.87 av.[c] | | | |

[a] *C.N.* = casein nitrogen.
[b] The same Jersey milk held for the times indicated at 4° C.
[c] Standard deviation, 0.056.

In the calculations for the first set, *i.e.*, for the unwashed deposits, the weight of dry complex was obtained by diminishing the total solids by the contained serum protein, by serum salts and lactose proportional to the free (not hydrate) water evaporated from the deposits, and by an additional 0.5% as an approximate correction for ether-extractable substances. The weight of calcium in the complex was obtained by diminishing the total calcium by calcium proportional to the free water evaporated.

In the calculations for the second set—the once-washed deposits—since analyses showed lactose, fat, and chloride to be negligible, the total solids value was diminished only by the contained serum protein, and no other corrections were used.

The colloid samples on which the third set of ratios is based were washed twice, because the once-washed deposits, despite negligible lactose by analysis, nevertheless showed slight yellowing in total solids determinations. The second washing seems permissible, because the results for Sets One and Two fail to

show any significant change in the nitrogen factor or the calcium : casein nitrogen ratio due to washing, and the complete analyses *(3)* similarly show no significant change in inorganic phosphorus. With these twice-washed samples, no yellowing in total solids determinations was noted. The dried colloids were colorless, glassy solids. The total solids values were corrected for contained serum protein as shown by analysis, as for the unwashed and once-washed deposits.

All of the skimmilks used for the preparation of the twice-washed deposits were submitted to sufficient preliminary centrifugation to remove those extremely large particles which apparently differ in composition from the rest *(3)*. These may represent from zero to about 15% of the total colloid. Although size classes are indicated in a general way (Table 2), close size fractionations were not attempted.

### CALCULATED FACTORS BASED ON COMPOSITION OF THE COMPLEX

Differential and direct analyses of the centrifugally separated casein colloid for total nitrogen, casein nitrogen, calcium, total phosphorus, and total solids indicate that the unit particle of serum-free casein colloid, *i.e.*, casein complex, may be considered as composed of calcium caseinate and tricalcium phosphate in the proportions of one phosphorus equivalent of the former to one-half mol of the latter; and that the weight ratio of organic phosphorus to nitrogen is 0.0563 *(3)*. The weight ratio of casein to nitrogen calculated from analyses of casein by Ramsdell and Whittier *(4)* is 6.45. This casein : nitrogen factor is also that used by deKadt and van Minnen *(1)*. Assuming the composition indicated, an aggregate of particles of casein complex containing 1 g. of nitrogen would be composed as follows:

| *Component* | *g.* |
|---|---|
| Casein | 6.45 |
| Calcium equivalent to organic phosphorus | 0.0727 |
| ½ $Ca_3(PO_4)_2$ equivalent to organic phosphorus | 0.2815 |
| Hydrogen replaced by calcium (subtracted) | −.0037 |
| Total | 6.8005 |

This total weight is a theoretical nitrogen factor for the complex, stripped of all calcium phosphate in excess of that assumed for the unit particle. The assumption that calcium in calcium caseinate is equivalent to the organic phosphorus is the same assumption made by deKadt and van Minnen *(1)*, and is based on various published experiments *(1, 4)*.

In the above analysis, the ratio of total calcium to nitrogen is 0.1817. Centrifugal analyses of many milk samples *(3)* give calcium : nitrogen ratios ranging from 0.181 to 0.232, and an average ratio of 0.206. Adjusting the tricalcium phosphate in the above analysis to make the calcium : nitrogen ratios 0.181, 0.206, and 0.232, the factors calculated are 6.798, 6.867, and 6.934. However, the separated colloid contains also about 1/15th mol of magnesium per mol of calcium. This does not affect the theoretical factor, 6.80, but, assuming all the magnesium to be present as $Mg_3(PO_4)_2$, it increases the experimental minimum,

average, and maximum complex : nitrogen factors to 6.814, 6.883, and 6.953, respectively.

According to the analysis given, the nitrogen factor for calcium caseinate is $6.45 + 0.0727 - 0.0037 = 6.519$. This is identical with the factor calculated from data by Ramsdell and Whittier *(4)*, who analyzed the washed complex, and then deducted tricalcium phosphate equivalent to the inorganic phosphorus from this analysis.

## DISCUSSION

*Comparison with factors calculated from data by others.* De Kadt and van Minnen *(1)* give analyses of successive liquid fractions and of unwashed deposits from which complex : casein nitrogen factors can be calculated. Handling these data as outlined here, for 11 sets of liquid samples, the average nitrogen factor obtained is 6.97, and the corresponding average calcium : nitrogen ratio is 0.201; for seven fractional deposits from three milks, the average nitrogen factor is 7.06, and the corresponding average calcium : nitrogen ratio, 0.221. These factors are about 1.5% higher than those here reported. It is presumed that de Kadt and van Minnen's milk was from Friesian cows. They used a Sharples Supercentrifuge.

*Importance of the numerical value for hydration, and possible errors arising from the corrections for nonproteinaceous solids: reliability of results by different methods.* The calculations based on analyses of liquid samples and on analyses of unwashed deposits both involve a numerical value, 0.52 g. per g., for hydration. Higher values for hydration would give higher nitrogen factors. The effect is appreciable. If a hydration of 1.00 g. per g. were assumed, for example, the average factor for liquids would be raised from 6.87 to 7.06; if the hydration were assumed to be zero, the factor would be lowered to 6.66. By comparison with the results for washed deposits, for which hydration is not a factor, it would appear that the true hydration is probably not far from the value used. The correction for included fatty substances (0.5% of the total solids removed) is an average correction, based on many ether extractions of unwashed dried deposits; but individual samples give ether extracts which vary widely, from zero to about 1%. Therefore, for individual samples, this correction may introduce an error of $\pm$ 0.5%. The corrections for serum total solids, in Equation 1 for liquid samples, and for unwashed deposits, assume that no more such solids, except serum proteins, are deposited as part of the colloid than would be contained in the free or serum water. It is possible that the amount of nonprotein serum solids in the deposits may be slightly more than this. The effect of excess serum proteins is eliminated in Equation 1, and is taken into account in the calculations for deposits.

The calculations based on analyses of washed deposits involve the assumption that the composition of the casein complex is unchanged by the washing. This assumption appears to be justified. Lactose, and presumably other serum solids, except serum proteins, are removed from the deposits by washing. The ether extract is reduced to a negligible value, as has been stated. Therefore, data for

washed colloids need be corrected only for retained serum proteins and, since this correction is based on analyses, it is presumed to be accurate. The results for washed deposits are, therefore, probably more reliable than those for liquid samples or unwashed deposits.

*Collected results.* Although arithmetic averages are given throughout this paper, such averages are not adequate, since a trend rather than a central tendency is involved.

In Figure 2 the various complex : casein nitrogen factors based on analyses

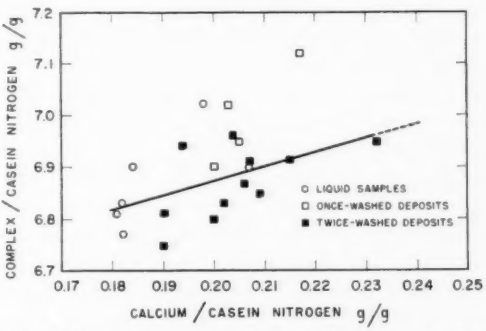


FIG. 2. Relationship of the colloid : casein nitrogen factor to the calcium : casein nitrogen ratio in the colloid, as determined by various methods.

of liquid samples, of once-washed deposits, and of twice-washed deposits, are collected and plotted against the corresponding calcium : casein nitrogen ratios. The deviations indicated may be in part attributable to experimental errors, but they also reflect the variability in composition of milks. The theoretical line, based on the composition of the complex as indicated by complete analyses, is drawn across the plot. The equation of this line is:

$$\text{Complex}/C.N. = 6.814 + (2.73)(\text{Ca}/C.N. - 0.181).$$

Here the constant, 6.814, is the calculated minimum factor for the complex, and the constant 2.73 represents g. of calcium phosphate plus g. of magnesium phosphate, per g. of calcium in excess of the experimental minimum. In the equation for the corresponding least squares line, giving equal weight to all the values, the constants are 6.820 and 3.90; omitting the values for the once-washed deposits, the constants are 6.823 and 2.70. In the first case, the standard error of estimate is 0.075, in the second case, 0.062. Considering the weight to be given the different sets of values, the relationship between the complex : nitrogen factor and the calcium : nitrogen ratio would appear to be adequately expressed by the equation:

$$\text{Complex}/C.N. = 6.82 + (2.7)(\text{Ca}/C.N. - 0.181). \qquad (2)$$

This equation gives minimum, average, and maximum factors, at the calcium : casein nitrogen ratios 0.181, 0.206, and 0.232, of 6.82, 6.89, and 6.96, respectively. The probable error is about 0.04, or 0.6%.

The calcium : casein nitrogen ratio for any milk, or fraction of the colloid, can be found by either direct or differential analysis of the separated colloid *(3)*. If only the analysis of the milk is known, the calcium : casein nitrogen ratio may be estimated, by assuming a serum calcium content (in the milk) of 0.038 g. per 100 g. For any particular milk sample of unknown analysis, the average factor 6.89 could be in error by roughly 1.3%.

Except in four cases, the results here presented are for Jersey milk. However, the values for the other milks and those calculated from de Kadt and van Minnen's data suggest that for all milks the nitrogen factor may be about the same as for Jersey milks, if not identical. It should also be noted that the results given do not apply to the very largest casein-containing particles in milk. In the preparation of the skimmilks, these large particles were, in most cases, and for all the washed deposits, purposely eliminated. Similarly, the results do not necessarily apply to the very smallest particles, representing 5 to 10% of the casein colloid: these particles were not removed in the centrifuging times used. As much as 99% of the colloid has, however, been centrifugally removed in other experiments.

Although the correlation here is primarily between the complex : nitrogen factor and the calcium : nitrogen ratio, a correlation between the calcium : nitrogen ratio and particle size has been shown *(3)*. In general, for any particular milk, the calcium content and, therefore, the nitrogen factor, will be greater for the large casein-containing particles than for the small ones.

*The trapped serum protein.* In all the calculations given in this paper, the serum protein deposited with the casein colloid on centrifuging is excluded. The amount of this serum protein may be twice or three times as much as the amount which can be accounted for as present in the entrained solvate liquid. The excess is carried down with the colloid, by sweeping action, or otherwise, and it cannot be removed from the deposit by washing. Whether the attachment occurs on deposition or exists in the liquid milk can not be decided from present data. In the calculations, it is assumed that the attachment occurs on deposition, and that none of the serum protein in deposits is properly to be considered a part of the complex.

*Nature of the actual colloidal particle in milk, and nitrogen factors for the total particle.* It is implied in the title of this paper, and stated in the introduction, that the nitrogen factor as calculated here refers only to the calcium caseinate–calcium phosphate portion of the casein-containing particles, or micelles, in milk. This substance may well represent a definite chemical complex; it may at least be considered the framework of the particle. These particles are spherical in shape and are very large, ranging in size upward from about 520 Å diameter. They may be visualized as loose aggregates of casein complex units, possibly coiled chains, to which hydrate water molecules are attached, and which are interpenetrated by solvate liquid containing salts, lactose, and serum proteins, in the same proportions in which these substances exist in the casein-free serum. The ratio of the volume of the total particle to the weight of contained dry complex is about 3.1 to 1 *(2)*. It can be calculated from this ratio and other data that

if the hydration is 0.52 g. per g., the remaining water present as solvate liquid should contain on the average about 0.124 g. of serum solids. Therefore, for the minimum and maximum factors, 6.82 and 6.96, for example, the corresponding minimum and maximum *colloid* : casein nitrogen factors are about 7.7 and 7.8, respectively. Again, the excess serum protein found in deposits is excluded.

The analyses of unwashed centrifugal deposits given in the previous paper *(3)* give over-all *total solids* : casein nitrogen ratios of 8.13 to 8.52, and when the serum protein in excess of that proportional to the serum water in the wet deposits is deducted from the total solids, the ratios 7.96 to 8.22 are obtained. Such factors depend upon the degree of compaction, however. By centrifuging at increasingly higher centrifugal forces, maximum compaction can be attained, and analyses of these deposits give ratios of total solids less trapped serum protein, *i.e.*, colloid : casein nitrogen factors, in satisfactory agreement with the calculated values, 7.7 to 7.8. Detailed discussion of such derived factors is premature here, since data on apparent specific volumes and voluminosity are involved. Determinations of these constants will be reported later. The purpose of this section is to make an explicit distinction between the total colloidal particle and the contained casein complex for which the factors here given are directly applicable.

## SUMMARY

1. Skimmilks were progressively depleted of casein colloids by centrifuging, and the supernatant liquids and deposited fractions analyzed. The dry weights of calcium caseinate–calcium phosphate complex contained in the separated colloids were calculated from the analyses. For many milks and many particle-size fractions the average ratio of dry weight of complex to casein nitrogen found is approximately $6.89 \pm 1.3\%$. This nitrogen factor is applicable to any milk sample of unknown composition.

2. The complex : nitrogen factor is shown to be correlated with the calcium : nitrogen ratio for the separated colloid, *i.e.*, with the ratio of complex bound calcium removed to casein nitrogen removed with the colloid. When this ratio is known, a more accurate nitrogen factor can be calculated by means of the statistically determined equation:

$$\text{Complex}/C.N. = 6.82 + (2.7)(\text{Ca}/C.N. - 0.181). \qquad (2)$$

The probable error when this equation is used is about 0.6%.

3. Since it has already been shown that the calcium : casein nitrogen ratio tends to increase with particle size, it follows that the nitrogen factor does also. The range of calcium : nitrogen ratios found experimentally is from 0.181 to 0.232; the corresponding range of nitrogen factors given by the equation is 6.82 to 6.96.

4. The experimental minimum nitrogen factor, 6.82, is in close agreement with a theoretical factor, 6.8005, calculated for a casein complex unit considered as composed of one mol of calcium caseinate and one-half mol of tricalcium phosphate. Adjusting this by adding trimagnesium phosphate in the proportion

in which magnesium is known to be present, the theoretical minimum factor becomes 6.814.

5. It is emphasized that the factors calculated are for the casein complex and not for the total colloidal particle, which also contains those serum solids associated with the solvate liquid. Colloid factors can be calculated from the complex factors by adding these additional solids. They range from approximately 7.7 to 7.8, in agreement with experimental results.

6. The nitrogen factors given do not apply to the very largest casein colloid particles in milk, and may not apply to the very smallest. The largest particles are known to differ in composition from the rest and were, therefore, in most cases purposely removed before the fractionations were made. The smallest particles were not all removed in the centrifuging times used, and no close fractionations of small particles only were attempted.

## REFERENCES

(1) De Kadt, G. S., and van Minnen, G. Condition of Casein and Salts, in Particular $Ca_3(PO_4)_2$, in Milk. *Rec. trav. chim.*, **62**: 257. 1943.

(2) Eilers, H. *Verslag Landbouwk.* Onderzoek., **50**: 1055. 1945. Eilers, H., Saal, R. N. J., and van der Waarden, M. *"Chemical and Physical Investigations of Dairy Products."* Elsevier Publ. Company, New York-Amsterdam. 1947.

(3) Ford, T. F., Ramsdell, G. A., and Landsman, Shirley G. Composition of the Casein-Containing Particles in Milk. *J. Dairy Sci.*, **38**: 843. 1955.

(4) Ramsdell, G. A., and Whittier, E. O. Composition of Casein in Milk. *J. Biol. Chem.*, **154**: 413. 1944.

# HEAT TOLERANCES OF JERSEY AND SINDHI-JERSEY CROSSBREDS IN LOUISIANA AND MARYLAND

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**Body temperatures and respiration rates were used to determine the heat tolerances of Jersey and Red Sindhi–Jersey crossbreds raised in Louisiana, and compared with those raised in Maryland. Lactating and nonlactating $F_1$ Sindhi-Jerseys were significantly less affected by exposures to hot environments than were Jerseys of comparable production levels. Nonlactating cows tested on cool days reacted differently from those tested on warmer days. Crossbreds are more easily acclimatized than purebreds.** *Editor.*

In an effort to utilize to greater advantage the dairy potentialities of the Gulf Coast Region of the United States, several southern Agricultural Experimental Stations, in cooperation with the Dairy Husbandry Research Branch of the USDA, have instituted experiments to determine the possibility of increasing the climatic adaptability of dairy cattle maintained there. One phase of the program has been carried out at the Iberia Livestock Experiment Station, Jeanerette, Louisiana, in cooperation with the Louisiana Agricultural Experiment Station; it involved the cross-breeding of Red Sindhi cattle (a milking strain of Zebu, native to India and Pakistan) with purebred Jerseys, in hopes of increasing the productivity of the Jersey progeny by adding some of the heat tolerance inherent in Indian cattle.

It is recognized that Zebu *(Bos indicus)* cattle are physiologically better able to withstand hot climates than are European-evolved *(B. taurus)* cattle. Differences between the two types of cattle are clearly evidenced by their responses to a variety of test situations. Brody and his coworkers *(1)* subjected *taurus* and *indicus* cattle at various times to environments outside the ranges of thermal neutrality. They report that the "comfort zone" (environmental temperature range in which no adjusting demands are made upon the physiological thermoregulatory mechanisms) for European cattle is between 30 and 60° F.; whereas, that for Indian cattle lies between 50 and 80° F. At environmental temperatures above 80° F., both types of cattle make adjustments, but the European cattle are forced to adjust quantitatively more than the Indian cattle. McDowell *et al. (5)* compared the rectal temperatures and respiratory rates of Jerseys and Red Sindhi–Jersey ($F_1$) crossbreeds in a standard hot environment, at different ages

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and stages of lactation; their results corroborate Brody's, in that they found a marked difference between the European cattle and the Indian-European crossbreds. Johnston and Frye *(3)* reported similar differences between Holsteins and Red Sindhi–Holstein crosses but, other than their work, few data have been gathered on dairy cattle outside of the temperate climatic zones, and still less on Indian-European crosses other than the $F_1$ half-breed.

The object of this report is to present data on heat tolerance comparisons, among different combinations of Jerseys and Red Sindhi–Jersey crossbreds raised in semitropical Louisiana, and to compare these results with those obtained with similar types of crosses, raised and tested in more temperate Maryland.

## METHODS

As a result of eight years of crossbreeding, the dairy herd at the Iberia Livestock Experiment Station contained purebred Jerseys, first-($F_1$) and second-($F_2$) generation half-breeds (Sindhi sire on Jersey dam for the $F_1$), and back crosses of the Sindhi and Jersey sires on the $F_1$ half-breed cows. A more complete enumeration of the herd composition has been reported elsewhere *(6)*, but only the above combinations will be considered in the ensuing report.

To measure heat tolerance, females in the herd were subjected routinely to a standard hot environment (105° F., 34 mm. Hg vapor pressure) in a climatic chamber for six hours at two-month intervals, from eight through 24 mo. of age, at one, two, four, and six months after parturition (second or later calving only), and when dry. During the six-hour test period, body temperatures and respiration rates were recorded hourly, and these measurements were assumed to reflect the degree of heat tolerance of the individual. For example, an individual having a high body temperature and respiration rate during the course of the test exposure was said to have a lower tolerance to heat than an animal with a lower body temperature and respiration rate.

Details of the climatic chamber and the testing procedure have been reported elsewhere *(5, 6)*; it need be repeated here only that the reaction of the animal is recorded in terms of the trapezoidal mean body temperature (or respiration rate) during the six-hour exposure period. Admittedly, the six-hour test routine used in this and related studies is limited, in that extrapolations to field conditions or long-term acclimatization must be made with extreme care. However, the authors feel that the test routine, if rigidly adhered to, does permit a rapid and reliable comparison of heat tolerance within and among breed groups and between station herds. Further, over-all results obtained in this study largely agree with those obtained in other studies *(4, 8)*, where longer test periods at similar or less drastic environmental conditions (more closely approximating field conditions) were used.

Since, at the time of this report, few data are available on lactating back-cross (¾ Sindhi–¼ Jersey; ¼ Sindhi–¾ Jersey) and $F_2$ animals, the comparisons between cows are confined to purebred Jerseys and $F_1$ Sindhi-Jersey crossbreds; the analyses of heifer groups (8 to 24 mo. old) include all five breed combi-

nations. Production levels of lactating cows refer to an F.C.M. average of the daily milk production for seven days preceding the test. Unfortunately, not enough purebred Red Sindhi animals were available for inclusion in the testing routine.

The analysis was designed to determine the effect of age, breed combination, initial environmental conditions (season of test), and level of milk production on body temperatures and respiratory rates of the test animals, before and during exposure to the standard hot climate. Statistical analyses were conducted according to the methods described in Snedecor *(7)* and Goulden *(2)*; the methods of fitted constants or weighted squares of means were used, where appropriate, to take into consideration the disproportionate subclass numbers in the various analyses.

## RESULTS

A. *Heifers.* Figures 1 and 2 show the means of initial body temperatures (hereafter designated as $T_o$), body-temperature reactions (designated as $T_m$), initial respiration rates ($R_o$), and respiration rate reactions ($R_m$), for each age

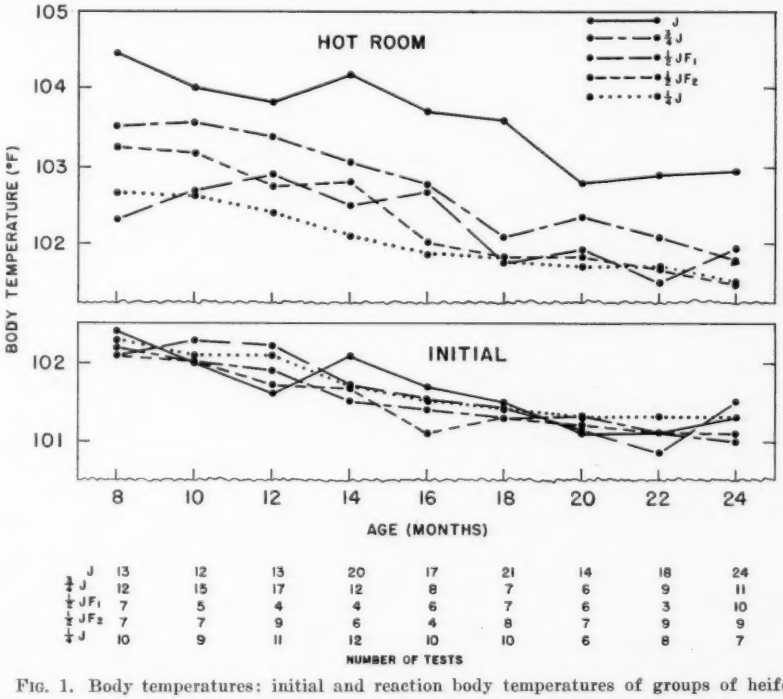


FIG. 1. Body temperatures: initial and reaction body temperatures of groups of heifers exposed to a standard hot atmosphere (see text). Numbers at foot of figure indicate the number of animals in each age group. (Jerseys: J; ¾ Jersey–¼ Sindhi: ¾J; ½ Jersey–½ Sindhi $F_1$: ½$JF_1$; ½ Jersey–½ Sindhi $F_2$: ½$JF_2$; ¼ Jersey–¾ Sindhi: ¼J.)

and breed group. The number of animals represented in each group is shown (bottom of Figure 1). The data indicate an inverse relationship between age and $T_o$, $T_m$, and $R_m$, but none between age and $R_o$. Further, there seems to be little indication of breed group differences with respect to $T_o$, but marked differences with respect to $R_o$, $T_m$, and $R_m$ (Figures 1 and 2). A summary of the results of statistical analysis of these data is presented (Table 1).

From the results, it appears that the degree of response (in terms of increased body temperatures and respiration rates) to the standard hot environment is inversely related to the amount of Sindhi blood in the individual. Paired comparisons (groups were paired on the basis of differences indicated in Figures 1 and 2) between the breed groups (Table 2) bear out these observations. Hence, if heat tolerance is defined as being inversely related to the body temperature or respiratory response to a hot environment, then the breed groups considered here can be ranked from lowest to highest heat tolerance as follows: Jerseys; ¾ Jersey–¼Sindhi; ½ Jersey–½ Sindhi ($F_1$ or $F_2$), and ¼ Jersey–¾ Sindhi.

B. *Dry cows.* The data gathered on dry cows (Jerseys and $F_1$ crossbreds only) follow essentially the same pattern as the heifer groups. Figure 3 (dry cows) summarizes the information available on this group, wherein the differences between breeds is significant with respect to $R_o$ ($t = 2.22$, $P < 0.05$), $T_m$ ($t = 10.61$,

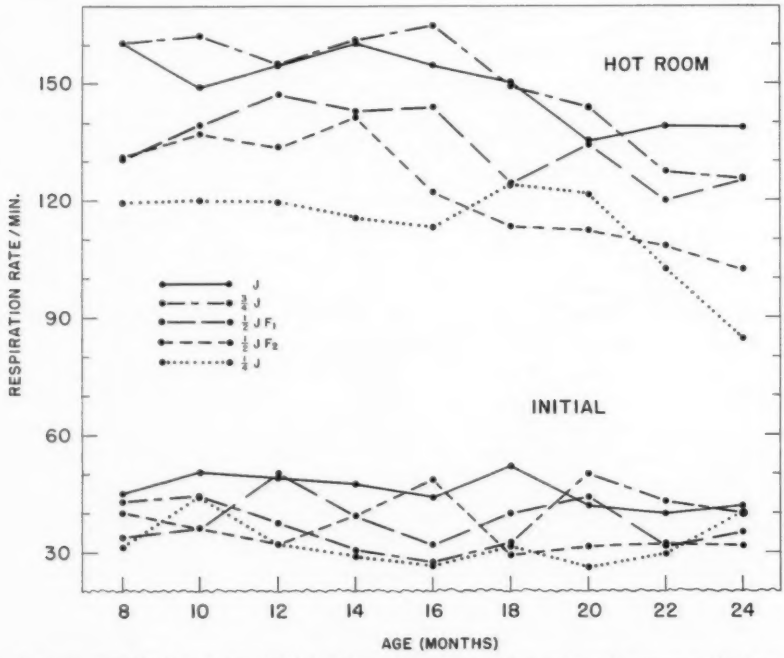


FIG. 2. Respiration rates: initial and reaction respiration rates of groups of heifers exposed to a standard hot atmosphere (see text). The number of animals in each group is the same as in Figure 1.

TABLE 1

*Analyses of variance of initial body temperatures ($T_o$) and respiration rates ($R_o$), and trapezoidal mean body temperatures ($T_m$) and respiration rates ($R_m$)*

| Source of variation | Degrees of freedom | Mean squares | | | |
|---|---|---|---|---|---|
| | | $T_o$ | $R_o$ | $T_m$ | $R_m$ |
| | | Heifers | | | |
| Ages | 8 | 8.13[b] | 334.28 | 15.00[b] | 5,227.86[b] |
| Breeds | 4 | 0.61 | 3,063.97[b] | 45.69[b] | 26,515.27[b] |
| Interaction | 32 | 0.25 | 292.66 | 0.63 | 477.56 |
| Error | 405 | 0.28 | 325.69 | 0.72 | 628.52 |
| | | Dry cows | | | |
| Subclasses | 3 | 0.66 | ..... | ..... | ..... |
| Breeds | 1 | ..... | 1,361.53[b] | 58.25[b] | 326.61 |
| Season of test | 1 | ..... | 4,686.32[b] | 3.61[b] | 7,625.72[b] |
| Interaction | 1 | ..... | 117.12 | 0.37 | 3,197.23[b] |
| Error | 83 | 0.25 | 213.52 | 0.58 | 341.08 |
| | | Lactating cows | | | |
| Breeds | 1 | 1.35 | 12,063.95[b] | 139.75[b] | 18,215.78[b] |
| Production levels | 3 | 0.58 | 186.37 | 3.02[b] | 204.94 |
| Interaction | 3 | 1.35[a] | 410.14 | 3.10[b] | 532.56 |
| Error | 249 | 0.37 | 237.27 | 0.70 | 231.67 |

[a] Significant at the 5% level of probability.
[b] Significant at the 1% level of probability.

TABLE 2

*Analyses of variance of paired breed groups with respect to body temperature and respiration rate responses ($T_m$ and $R_m$)*

| Source of variation | D.F. | M.S. | D.F. | M.S. | D.F. | M.S. |
|---|---|---|---|---|---|---|
| | | | $T_m$ | | | |
| Groups compared | | Jersey and ¾ Jersey[a] | | ¾ Jersey and $F_1 + F_2$[b] | | $F_1 + F_2$ and ¼ Jersey[c] |
| Breeds | 1 | 41.37** | 1 | 10.04** | 1 | 3.26* |
| Ages | 8 | 10.26** | 8 | 9.03** | 8 | 5.22** |
| Interaction | 8 | 0.99 | 8 | 0.30 | 8 | 0.27 |
| Error | 231 | 0.86 | 197 | 0.68 | 183 | 0.55 |
| | | | $R_m$ | | | |
| | | Jersey and $F_1$ | | $F_1$ and $F_2$ | | $F_1 + F_2$ and ¼ Jersey |
| Subclasses | ..... | ..... | 17 | 1,162.63 | ..... | ..... |
| Breeds | 1 | 9,360.97** | ..... | ..... | 1 | 10,000.11** |
| Ages | 8 | 1,662.57** | ..... | ..... | 8 | 2,137.37** |
| Interaction | 8 | 346.87 | ..... | ..... | 8 | 748.86 |
| Error | 186 | 455.60 | 100 | 767.89 | 183 | 798.52 |

* Significant at the 5% level of probability.
** Significant at the 1% level of probability.
[a] ¾ Jersey, ¼ Sindhi.
[b] First- and second-generation Sindhi-Jersey half-breeds, respectively.
[c] ¼ Jersey, ¾ Sindhi.

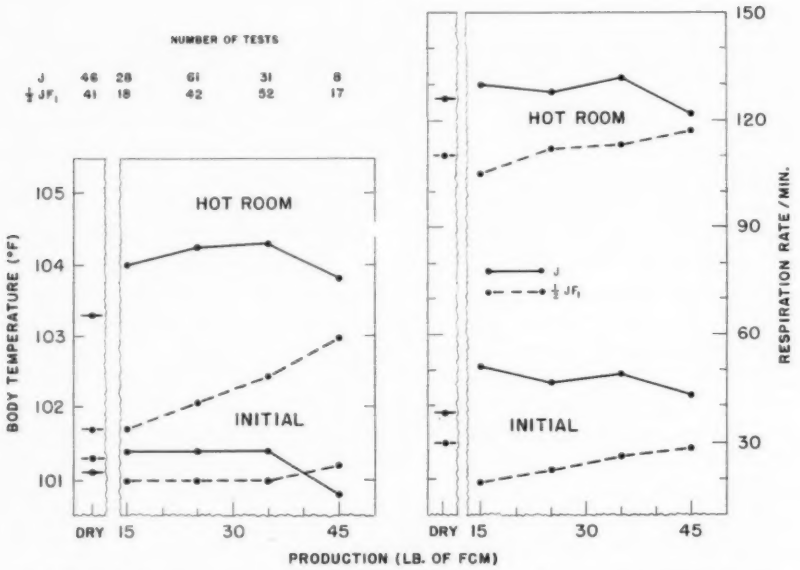


FIG. 3. Body temperatures and respiration rates: initial and mean responses of Jersey and Sindhi–Jersey $F_1$ half-breed cows to a standard hot atmosphere (see text.) Numbers at top of figure indicate the number of Jerseys (J) and cross-breds (½ JF) in each production group.

$P < 0.01$), and $R_m$ ($t = 3.78$, $P < 0.01$), but not with respect to $T_o$ ($t = 1.16$, $P > 0.05$).

Since dry cows were tested during various parts of the year, it was possible to divide them roughly into "season-of-test" groups. The dry-bulb temperature in the test chamber at 7 A.M. (before the heat was turned on) was used as a guide in separating the "winter" from the "summer" tests: temperatures of 74° F. or less (down to as low as 46° F.) established the winter tests; whereas, the range of starting chamber temperatures of the summer tests was from 75 to 88° F. Results of a statistical analysis of the differences with respect to breeds and season of test are shown (Table 1).

It is not surprising to note no detectable difference in $T_o$ with respect to season of test, since most of the initial chamber temperatures were well within the range of thermal neutrality (50 to 80° F.). Minor compensations within this range were apparently accomplished via the respiratory system, which accounts for the significant differences in $R_o$. It is unlikely that the lower respiratory rates of the crossbreds signify a more efficient homeostatic mechanism, since the actual difference between summer and winter respiratory rates (Table 3) is essentially the same for each breed group. On the other hand, these data tend to support Brody's hypothesis *(1)* of a basic difference between breeds in the amount of body heat to be disseminated (*i.e.*, a difference in metabolic rates).

With respect to $T_m$, the variance due to season of test is much less than that due to breed group (Table 1). From this it can be assumed that, although season of test is important in the final assessment of a group's heat tolerance, it is probably not so important as to invalidate the breed-group differences established in this study.

Somewhat different results are obtained in the analysis of $R_m$ (Table 1): here, differences due to breeds are not significant, while those attributable to season of test are. In view of the fact that the $R_m$ of one breed group decreased from one season to the other, while it increased in the other breed, it is not surprising

TABLE 3

*Dry cows: mean values of initial body temperatures ($T_o$) and respiration rates ($R_o$), and trapezoidal mean body temperatures ($T_m$) and respiration rates ($R_m$) of nonlactating Jersey and $F_1$ crossbred cows*

| Season of test[a] | No. of records | $T_o$ | $R_o$ | $T_m$ | $R_m$ |
|---|---|---|---|---|---|
| | | | Jerseys | | |
| Winter | 16 | 100.9 | 38.3 | 102.9 | 136.9 |
| Summer | 30 | 101.3 | 52.1 | 103.5 | 119.4 |
| | | | $F_1$ Crosses | | |
| Winter | 11 | 101.2 | 26.9 | 101.5 | 103.2 |
| Summer | 30 | 101.3 | 45.6 | 101.8 | 112.2 |

[a] See text for explanation of "season."

that the breed × season-of-test interaction is highly significant. The inconsistency of such results might possibly be eliminated by a larger sample.

C. *Lactating cows.* Data on lactating cows were analyzed with respect to breed (Jersey and $F_1$ crossbreds) and production levels (F.C.M. average of daily production during the seven days immediately preceding a test exposure). These data are presented (Figure 3); a summary of the statistical analyses is shown in Table 1.

In view of the significant production × breed interaction in both $T_o$ and $T_m$ (Table 1), it is necessary that these data be carefully evaluated before any conclusions are drawn from them. Body-temperature responses of Jerseys at the highest production level are less than at lower production levels (Figure 3); this is quite different from results obtained with the crossbreds, and is also at variance with data published by McDowell *(5)*. However, it will be noted that the initial body temperatures of these high-production Jerseys were markedly "out of line" with respect to the other $T_o$ data (which in turn would affect the $T_m$). Since this statistic was obtained from an unusually small group (only eight animals, less than half of the next smallest group), it is suggested that the 45-lb. Jersey class represents an aberrant sample, and that the significant interaction would be eliminated by a larger group-size.

It is interesting to note that if one considers the change in body temperature resulting from the test exposure $(T_m\text{-}T_o)$, one gets an orderly increase in response, directly related to production (Jersey, 2.6, 2.8 2.9, 3.1° F. at 15-, 25-, 35-, and

45-lb. production classes, respectively), despite the lower $T_o$ and $T_m$ at the 45-lb. class. Similar data for crossbreeds (0.7, 1.1, 1.4, 1.8° F. at 15-, 25-, 35-, and 45-lb. production classes, respectively) show the same orderly increase in response directly related to production, but at a considerably lower level. In contrast, increases in respiratory rates ($R_m$-$R_o$) are about the same for both breed groups (despite the significantly lower initial values of the crossbreeds) and do not seem to be related to production levels: Jerseys, 79, 81, 83, and 80 resp/min; half-breeds, 76, 79, 76, and 79 resp/min, at 15-, 25-, 35-, and 45-lb. production classes, respectively.

These data serve to re-emphasize the basic physiological differences between the two breeds, with respect to heat production and metabolic rates. It is interesting also to note (Figure 3) that both groups are near the ceiling of their respiratory capabilities wherein further rate adjustments are not possible, and that the ceilings of the two groups are at different levels.

## DISCUSSION

Since the routine test procedures, breed groups, and analytical methods used were essentially the same as those employed by McDowell *et al.* (*5*) at Beltsville, a comparison of both sets of results might be used as a guide in evaluating geographical differences. The Beltsville station is located in eastern United States at approximately 39° N. latitude; whereas, the Jeanerette station is located along the Gulf Coast of southern United States at approximately 30° N. latitude. Aside from relatively minor management and technical (chamber-operation) differences between the two stations, the major point to be considered is the physiological differences between the animals, resulting from being raised under quite different environmental conditions. One would expect that the Louisiana cattle would be less markedly affected by the test exposure, owing, perhaps, to a certain degree of acclimatization.

Data from the two stations are presented (Table 4), for comparison. In general, the younger Jeanerette heifers tend to show slightly higher body temperatures and respiration rates than do the Beltsville animals, regardless of breed. This discrepancy tends to disappear in the older heifer groups, and is apparently completely gone in the initial body temperatures and respiration rates of the lactating and dry cows.

It is interesting to note that the Jeanerette cows show consistently lower $R_m$'s than do the Beltsville cows, this in spite of the fact that Jeanerette Jersey $T_m$'s are somewhat higher than those of Beltsville Jerseys. These results suggest that the Jerseys develop some degree of adaptation to the warmer climate of Jeanerette; the adaptation manifests itself by a much lower respiratory rate during periods of heat stress, at a cost of only a slightly higher body temperature. On the other hand, crossbreds raised under both environmental conditions (Jeanerette and Beltsville) show a much greater adaptation to the warm climate, as evidenced by lower body temperature responses despite lower respiratory rates, at all levels of production.

A comparison of the tables of analyses of the Jeanerette data with those obtained at Beltsville (Table 5) reveals similarities all the more startling, in view of the adaptation differences in the animals just mentioned. Indeed, with respect to body temperatures, the only real difference is that the breed × production level interaction in $T_o$ was significant at Jeanerette, but not at Beltsville; in all other cases, the significant differences obtained with the two sets of data coincide.

TABLE 4

*Comparison of the Beltsville[a] and Jeanerette means of initial body temperatures ($T_o$) and respiration rates ($R_o$), and trapezoidal mean body temperatures ($T_m$) and respiration rates ($R_m$)*

| Groups | $T_o$ | | $R_o$ | | $T_m$ | | $R_m$ | |
|---|---|---|---|---|---|---|---|---|
| | Jean. | Belts. | Jean. | Belts. | Jean. | Belts. | Jean. | Belts. |
| | | | | Heifer age groups | | | | |
| | (° F.) | | (resp/min) | | (° F.) | | (resp/min) | |
| 8-mo. Jersey | 102.4 | 101.8 | 44.5 | 36.2 | 104.5 | 103.4 | 160.1 | 152.8 |
| Crosses[b] | 102.1 | 101.9 | 34.3 | 32.5 | 102.3 | 102.3 | 130.3 | 134.9 |
| 10-mo. Jersey | 102.0 | 101.6 | 50.3 | 34.7 | 104.0 | 103.6 | 149.5 | 152.0 |
| Crosses | 102.3 | 101.8 | 36.0 | 35.7 | 102.7 | 102.5 | 139.7 | 135.5 |
| 12-mo. Jersey | 101.6 | 101.3 | 48.8 | 34.1 | 103.8 | 103.0 | 156.0 | 150.8 |
| Crosses | 102.2 | 101.5 | 50.0 | 32.3 | 102.9 | 102.3 | 147.6 | 138.8 |
| 14-mo. Jersey | 102.1 | 101.4 | 47.8 | 40.4 | 104.2 | 103.1 | 160.7 | 153.8 |
| Crosses | 101.7 | 101.4 | 39.0 | 25.5 | 102.5 | 102.2 | 142.8 | 145.7 |
| 16-mo. Jersey | 101.7 | 101.3 | 44.1 | 38.0 | 103.7 | 102.8 | 154.7 | 149.0 |
| Crosses | 101.6 | 101.4 | 31.7 | 30.7 | 102.7 | 101.9 | 143.9 | 131.1 |
| 18-mo. Jersey | 101.5 | 101.3 | 52.0 | 37.7 | 103.6 | 102.7 | 151.2 | 150.3 |
| Crosses | 101.4 | 101.3 | 40.0 | 29.7 | 101.7 | 102.0 | 124.8 | 133.6 |
| 20-mo. Jersey | 101.1 | 101.3 | 42.1 | 34.7 | 102.8 | 102.5 | 135.8 | 150.9 |
| Crosses | 101.1 | 101.4 | 44.0 | 26.0 | 101.9 | 101.9 | 134.2 | 133.3 |
| 22-mo. Jersey | 101.1 | 101.3 | 40.2 | 28.6 | 102.9 | 102.7 | 139.2 | 144.9 |
| Crosses | 100.7 | 101.4 | 30.7 | 30.3 | 101.5 | 102.1 | 119.8 | 133.2 |
| | | | | Dry cows | | | | |
| Jerseys | 101.1 | 101.1 | 47.3 | 35.3 | 103.3 | 102.9 | 125.5 | 142.0 |
| Crosses | 101.3 | 101.1 | 40.6 | 31.0 | 101.7 | 102.1 | 109.8 | 136.0 |
| | | | | Lactating cows, production levels | | | | |
| [c]15, 20 lb. Jersey | 101.4 | 101.4 | 50.9 | 39.5 | 104.0 | 103.5 | 130.0 | 135.1 |
| Crosses | 101.0 | 101.2 | 28.9 | 41.3 | 101.7 | 102.8 | 104.8 | 141.9 |
| 25, 30 lb. Jersey | 101.4 | 101.3 | 46.8 | 36.7 | 104.2 | 103.6 | 127.8 | 134.4 |
| Crosses | 101.0 | 101.5 | 32.8 | 41.5 | 102.1 | 103.2 | 112.3 | 148.4 |
| 35, 40 lb. Jersey | 101.4 | 101.5 | 49.2 | 44.0 | 104.3 | 104.1 | 131.8 | 138.7 |
| Crosses | 101.0 | 101.5 | 36.7 | 38.9 | 102.4 | 102.9 | 113.2 | 145.8 |
| 45, 50 lb. Jersey | 100.7 | 101.5 | 43.0 | 51.2 | 103.8 | 104.4 | 122.8 | 143.3 |
| Crosses | 101.2 | 101.5 | 38.6 | 40.9 | 103.0 | 103.1 | 117.5 | 144.1 |

[a] Data adapted from McDowell *et al.* (5).
[b] Crosses refer to Sindhi-Jersey $F_1$ crossbreds.
[c] The Jeanerette data were analyzed in terms of 15-, 25-, 35-, and 45-lb. production levels; whereas, those at Beltsville were in terms of 20, 30, 40, and 50 lb.

Some station differences in respiratory rates are apparent. For example, with respect to $R_o$, differences attributable to breeds at Jeanerette (in the lactating cow groups) were statistically significant, but not at Beltsville; conversely, Beltsville levels-of-production groups showed significant differences, whereas those at Jeanerette did not. When sufficient data have been gathered to permit an adjustment for season of test, it is expected that these discrepancies will disappear.

TABLE 5

*Comparison of the analyses of data gathered at Beltsville[a] with those obtained at Jeanerette*

| Source of variation | $T_o$ | | $R_o$ | | $T_m$ | | $R_m$ | |
|---|---|---|---|---|---|---|---|---|
| | Jean. | Belts. | Jean. | Belts. | Jean. | Belts. | Jean. | Belts. |
| | | | Heifers | | | | | |
| Ages | ** | ** | n.s. | n.s. | ** | ** | ** | n.s. |
| Breeds[b] | n.s. | n.s. | ** | ** | ** | ** | ** | ** |
| Interaction | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| | | | Dry cows | | | | | |
| Breeds | n.s. | n.s. | ** | * | ** | ** | n.s. | n.s. |
| | | | Lactating cows | | | | | |
| Breeds | n.s. | n.s. | ** | n.s. | ** | ** | ** | ** |
| Production levels | n.s. | n.s. | n.s. | * | ** | ** | n.s. | n.s. |
| Interaction | * | n.s. | n.s. | n.s. | ** | ** | n.s. | n.s. |

* Differences significant at the 5% level of probability.
** Differences significant at the 1% level of probability.
n.s. Differences not statistically significant.
[a] Data adapted from McDowell *et al.* *(5)*.
[b] Only Jersey and $F_1$ half-breeds are represented in the Beltsville heifer data.


## SUMMARY

Jerseys and various crosses of Red Sindhi and Jersey ($F_1$, $F_2$, back-cross to Jersey and Sindhi) were subjected at regular intervals to a standard hot environment of 105° F. at 34 mm. Hg (92° F. wet bulb), for six hours in a climatic control chamber. The relative tolerances of the different breed combinations were assessed, using body temperatures and respiration rates as indices of heat tolerance. This paper describes the results of the test exposures, with respect to ages and stages of lactation of the animals, and affords a comparison between the different breed combinations used. Further, the results obtained at Jeanerette, Louisiana, are compared with those obtained at Beltsville, Maryland, so as to determine whether there are indications of long-term climatic adaptation.

The data indicate an inverse relationship between age (up to 24 mo.) and reactions to the standard hot environment, and marked differences in the reactions of the breed groups tested. In general, the degree of tolerance to the test hot environment is directly related to the amount of Sindhi blood in the animal, so that the breed groups tested could be ranked (from lowest to highest heat tolerance) as follows: purebred Jersey; ¾ Jersey–¼ Sindhi; ½ Jersey–½ Sindhi ($F_1$ and/or $F_2$), and ¼ Jersey–¾ Sindhi.

In the cow groups, both dry and lactating, $F_1$ Sindhi-Jerseys were significantly less affected by the test exposures (in terms of body temperatures and respiration rates) than were Jerseys of comparable levels of production. The level of production in itself seemed to be directly related to the body temperature reactions within breed groups. Dry cows tested on "cool" days (where environmental temperature at start of test exposure was less than 75° F.) reacted significantly differently from those tested on "hot" days, indicating a seasonal difference in degree of acclimatization.

The reactions of heifers tested at Jeanerette, Louisiana, were very much like those of similar breed groups exposed to the same treatment at Beltsville, Maryland. On the other hand, the body temperature reactions of the Jeanerette $F_1$ half-breed cows (lactating and dry) were markedly lower than the Beltsville $F_1$ half-breeds. The respiratory reactions of the Jeanerette cows, both $F_1$ half-breeds and purebred Jerseys, were lower than comparable breed groups at Beltsville. These results suggest that some degree of acclimatization to a warmer environment is evident in the Jeanerette cows, and that the crossbreds are more markedly acclimatized than are the Jerseys at the same station.

## ACKNOWLEDGMENTS

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## REFERENCES

(1) Brody, S. Climatic Physiology of Cattle. *J. Dairy Sci.*, **39**: 715. 1956.

(2) Goulden, C. H. *Methods of Statistical Analysis.* 2nd ed. Wiley and Co., N. Y. 1952.

(3) Johnston, J. E., and Frye, J. B., Jr. Comparative Heat Tolerance of Holstein and Crossbred Red Sindhi–Holstein Heifers when Exposed to Elevated Temperatures and Humidities. *J. Dairy Sci.*, **36**: 585. 1953.

(4) Kellgren, H., Johnston, J. E., Frye, J. B., Jr., and Green, B. Response of Holstein-Friesian, Jersey, Red Sindhi–Holstein ($F_1$), and Red Sindhi–Jersey ($F_1$) Bulls to a Simulated Tropical Climate. *J. Dairy Sci.*, **37**: 667. 1954.

(5) McDowell, R. E., Lee, D. H. K., Fohrman, M. H., Sykes, J. F., and Anderson, R. A. Rectal Temperature and Respiratory Responses of Jersey and Sindhi-Jersey ($F_1$) Crossbred Females to a Standard Hot Atmosphere. *J. Dairy Sci.*, **38**: 1037. 1955.

(6) Schein, M. W., Hyde, C. E., Cathcart, S. L., and Fohrman, M. H. Dairy Cattle Research at the Iberia Livestock Experiment Station. USDA, Agr. Research Serv., *Bull.* ARS-52-4. 1955.

(7) Snedecor, G. W. *Statistical Methods.* 4th ed. Iowa State College Press, Ames. 1946.

(8) Worstell, D. M., and Brody, S. Environmental Physiology. XX. Comparative Physiological Reactions of European and Indian Cattle to Changing Temperature. Univ. Missouri Agr. Expt. Sta., *Research Bull.* 515. 1953.

# EFFECT OF AN ELECTRIC CURRENT ON THE EFFICIENCY OF HOMOGENIZATION OF ULTRASONICALLY IRRADIATED MILK[1]

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**The effect of ultrasonic irradiation on the efficiency of homogenization while direct current passed through milk was studied. The efficiency of homogenization increased with current increase to a maximum and then decreased with a further increase in current. Tests were run using a metal tube for a container with a center electrode. The polarity of the container and the center probe was reversed. Irradiating milk in the ultrasonic transducer bowl, when the platinum electrode center probe was negative, improved homogenization efficiency.** *Editor.*

High-frequency inaudible sound waves, "ultrasonics,"[3] are used to produce many phenomena pertaining to the realm of colloid chemistry, as first shown in 1927 by Wood and Loomis *(13)*. Later, it was found that substantially the same phenomena may be obtained with low-frequency sound waves, provided—and this is the fundamental difference—their intensity is sufficiently high.

Sound waves may bring about many phenomena, such as peptization, the mutual emulsification of two bulk liquid phases, the disintegration of certain solids, the depolymerization of high-molecular substances, coagulation of certain suspensions, production of heating effects, and chemical reactions, destruction of bacteria, and stimulation of plant growth. The pioneering attempts by Chambers and Gains *(5)*, Harvey and Loomis *(8)*, and Liu and Yen *(10)*, to effect certain physical-chemical and biological changes in milk and in various manufactured milk products, stimulated this study. Attention was directed to investigating the effect of an ultrasonic field upon fat dispersion (homogenization). Not only did the work of these early investigators indicate certain practical applications but work of more recent researchers gave further impetus to this interest *(1-4, 6, 9, 11, 12)*. Much of the early work was done prior to the availability of focusing ultrasonic transducer elements, whereby a considerably higher energy intensity level can be transmitted to the substance under investigation. Consequently, if there is a threshold intensity level necessary to effect biophysical changes, it is well to reinvestigate some of the earlier reports.

The review of the literature pertaining to the properties of ultrasonic energy and their effects on matter reveal the following principal characteristics: *(a)* Ultrasonic energy of sufficient intensity can cause emulsification; *(b)* emulsification

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[3] In the early literature concerning this subject, the term "supersonics," and occasionally "suprasonics," was used by some authors instead of "ultrasonics." Since about 1935, the term ultrasonics is most frequently used.

occurs only when cavitation is present; *(c)* cavitation by ultrasonic energy depends upon the presence of a dissolved gas phase, acting as nuclei for centers of cavitation—the kinds of gases are not important; *(d)* the smaller the gas bubble, the greater the cavitation. Large bubbles act as a cushioning medium and decrease the collapsing pressure; *(e)* a gas barrier is an efficient absorber of ultrasonic energy if generated within the gas; *(f)* ultrasonic energy produces degassing of the substances being radiated; *(g)* emulsification is particularly strong at the walls of the containing vessel, and to a lesser extent within the interior of the liquid; *(h)* ultrasonic energy can be employed to promote coagulation; *(i)* coagulation occurs within the liquid and not at the container walls, *(j)* emulsification precedes coagulation, but eventually coagulation and emulsification occur simultaneously, seemingly at the same rate.

The purpose of the experiment was to determine the effect of ultrasonic irradiation on homogenization, while simultaneously passing direct current through whole milk.

## EXPERIMENTAL PROCEDURE

Pasteurized whole milk having approximately a 3.8% butterfat content was used. In all cases, the milk was preheated to 150° F. ± 2° for 5 min. prior to ultrasonic treatment and for control samples. This constant temperature was selected to eliminate the temperature variable and to treat milk at the temperature commonly used in commercial homogenization.

*Ultrasonic apparatus.* The basic generator and transducer assembly consisted of a Brush, Model BU-204, 250W (Figure 1). The basic ultrasonic and trans-

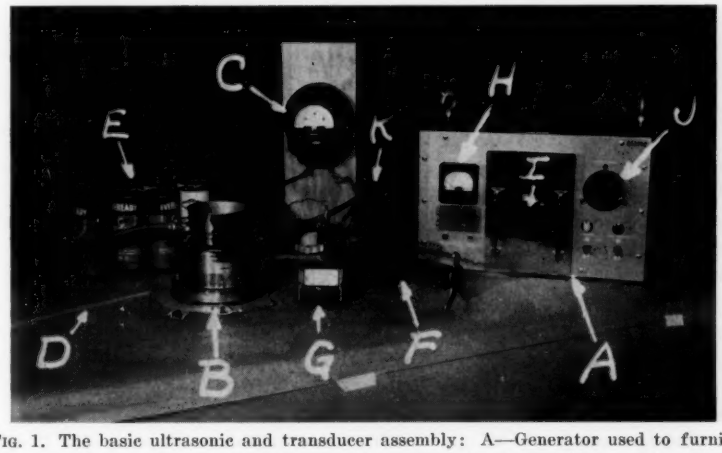



FIG. 1. The basic ultrasonic and transducer assembly: A—Generator used to furnish the ultrasonic energy, B—Barium titanate transducer assembly, C—Watt-hour meter for determining input to generator, D—Thermometer, E—Dry cells for d.c. source through milk, F—Rheostat for regulating the current through the milk, G—Milliammeter for measuring current through milk, H—Ammeter for measuring current strength of the generator, I—Generator frequency control, J—Generator output control, K—Coaxial cable between generator and transducer.

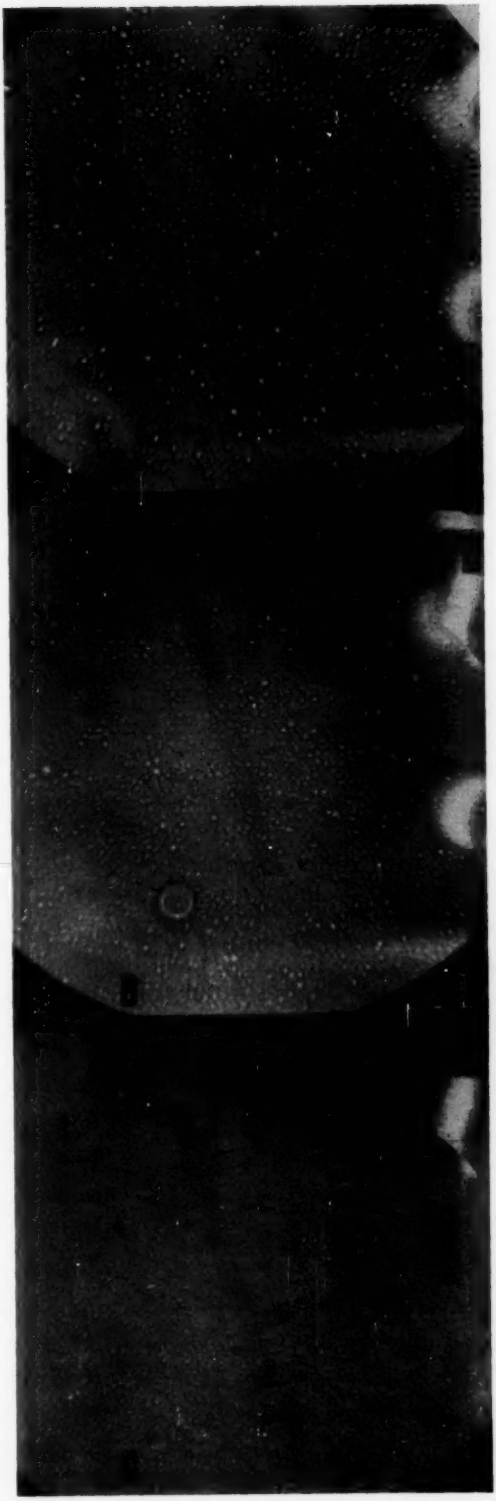
FIG. 2. Disassembled transducer: Cooling coils (A) are coiled around the inside of the upper section (B), which assembles into the transducer housing (C), barium titanate transducer element (D).

ducer assembly: A—Generator used to furnish the ultrasonic energy, B—Barium titanate transducer assembly, frequency of 430 kc, C—Watt-hour meter for determining input to generator, D—Thermometer, E—Dry cells for d.c. source through milk, F—Rheostat for regulating the current through the milk, G—Milliammeter for measuring current through milk, H—Ammeter for measuring current strength of the generator, I—Generator frequency control, J—Generator output control, K—Coaxial cable between generator and transducer. Figure 2 shows disassembled transducer. Cooling coils (A) are coiled around the inside of the upper section (B), which assembles into the transducer housing, (C), barium titanate transducer element (D). The output of the generator (M.A.), and the current strength through the milk sample (m.a.), are reported in milliamperes.

*Methods of evaluation.* Due to the small sample size used, the U.S.P.H.S. test could not be used. Instead, a test based on gravity separation was devised. Tubes of treated samples, along with untreated controls, were observed for evidence of cream-line after standing for 48 hr. at 40° F. A sample was considered homogenized only if no trace of cream-line was evident at the end of this period. Also, several treated and untreated specimens were microscopically studied for comparative fat-globule size, clumping, and dispersion (Figure 3—Photomicrograh of the fat globules in milk: A—Untreated, B—Ultrasonically radiated, C—Ultrasonically radiated in presence of an electric current. 1400×).

*Performance index.* The volume (ml.) of milk homogenized was compared to the time (min.) required to produce homogenization, and was reported hereinafter as the "efficiency factor" (ml/min).

*Ultrasonic treatments.* Procedures A, B, and C were designed to investigate the effect of the passage of an electric current through the sample simultaneously with ultrasonic irradiation. Procedure D was designed to determine the effect of the passage of an inert gas through the sample simultaneously with ultrasonic irradiation. Since the passage of an electric current through milk produces an



evolution of gases, Procedure D was incorporated to learn whether the effect of an electic current on homogenization was peculiar to the presence of an inert gas, or to the passage of an electric current.

*Procedure A.* Three variables were studied; namely, sample size, magnitude of the current through the sample, and output current of the generator. Milk was irradiated in a glass test tube, both with and without the passage of an electric current through the sample. Distilled water was used in the transducer, as a transmission medium between the transducer element and the test tube. The water temperature was maintained at 150° F. ± 5° by passing tap water through the cooling coils of the transducer. The test tubes were positioned in the transducer with their bottoms at the focal point of the ultrasonic beam. The electrodes were platinum wires. The radiation vessels were Pyrex test tubes (12 × 150 mm.).

Results of these trials are given (Table 1). For all sample sizes and for all generator output currents, the efficiency factor increased with an increase in direct current through the sample. The efficiency factor increased with an increase in the generator output current for every sample size and for each variation in the current, through the sample being irradiated.

FIG. 3. Photomicrograph of the fat globules in milk: A—Untreated, B—Ultrasonically radiated, C—Ultrasonically radiated in presence of an electric current. (1400×)

TABLE 1

*Effect of an electric current on the efficiency of homogenization by ultrasonic irradiation of milk, in a glass tube containing two platinum electrodes*

(Procedure A)

| Sample size | Current passed through sample | Homogenization efficiency factor | | |
|---|---|---|---|---|
| | | Generator output 200 M.A. | 175 M.A. | 150 M.A. |
| *(ml.)* | *(m.a.)* | *(ml/min)* | | |
| 10 | 0.0 | 1.11 | 1.00 | 0.66 |
| 10 | 5.0 | 3.33 | 2.50 | 1.00 |
| 10 | 10.0 | 3.33 | 3.33 | 1.11 |
| 10 | 15.0 | 3.33 | 3.33 | 1.25 |
| 20 | 0.0 | 1.33 | 1.17 | 0.95 |
| 20 | 5.0 | 2.00 | 1.66 | 1.33 |
| 20 | 10.0 | 2.22 | 1.66 | 1.54 |
| 20 | 15.0 | 2.50 | 2.00 | 1.80 |
| 30 | 0.0 | 0.85 | 0.80 | 0.65 |
| 30 | 5.0 | 1.50 | 1.11 | 0.78 |
| 30 | 10.0 | 1.57 | 1.15 | 0.81 |
| 30 | 15.0 | 1.76 | 1.25 | 0.83 |

*Procedure B.* Milk was irradiated in an aluminum test tube, with and without the passage of an electric current through the sample. The aluminum tube served as one electrode, and a platinum probe, centrally located, served as the other. As in Procedure A, distilled water was used in the transducer as a transmission medium between the transducer element and the radiation tube. The water temperature was maintained at 150° ± 5°. The radiation tube was positioned in the transducer as in *Procedure A*. Two variables, current through the sample and polarity of the tube and probe, were studied. The intensity of irradiation (output current of generator M.A.) and the sample size were kept constant.

The experimental data of these trials are reported (Table 2). The homogenization efficiency factor increased with an increase in the current (m.a.) to a maximum value, followed by a decrease in efficiency factor with an increase in current.

TABLE 2

*Effect of an electric current on the efficiency of homogenization, by ultrasonic irradiation of milk in a metallic tube containing a central platinum electrode*

(Procedure B)

| Sample size | Current passed through sample | Homogenization efficiency factor[a] | |
|---|---|---|---|
| | | Tube (+), Probe (−) | Tube (−), Probe (+) |
| *(ml.)* | *(m.a.)* | *(ml/min)* | |
| 8 | 0.00 | 1.60 | 2.00 |
| 8 | 5.00 | 4.00 | 4.00 |
| 8 | 10.00 | 3.20 | 4.00 |
| 8 | 15.00 | 4.00 | 4.00 |
| 8 | 20.00 | 2.70 | 2.70 |

[a] Generator output to transducer: 200 M.A.

TABLE 3

*Effect of an electric current on the efficiency of homogenization by ultrasonic irradiation of milk, in the transducer bowl containing a central platinum electrode*

(Procedure C)

| Generator output | Current passed through sample[a] | Time to effect homogenization | | Homogenization efficiency factor | |
|---|---|---|---|---|---|
| | | Probe (−) | Probe (+) | Probe (−) | Probe (+) |
| *(M.A.)* | *(m.a.)* | *(min.)* | *(min.)* | *(ml/min)* | *(ml/min)* |
| 150 | 0.0 | 220[b] | 240[b] | 0.00 | 0.00 |
| 150 | 9.0 | 220[b] | 240[b] | 0.00 | 0.00 |
| 150 | 18.0 | 220[b] | 240[b] | 0.00 | 0.00 |
| 150 | 30.0 | 240[b] | 240[b] | 0.00 | 0.00 |
| 175 | 0.0 | 180 | 240[b] | 1.95 | 0.00 |
| 175 | 9.0 | 120 | 240[b] | 2.91 | 0.00 |
| 175 | 18.0 | 110 | 240[b] | 3.18 | 0.00 |
| 175 | 30.0 | 105 | 240 | 3.33 | 1.45 |
| 200 | 0.0 | 150 | 240 | 2.33 | 1.45 |
| 200 | 9.0 | 65 | 225 | 5.38 | 1.55 |
| 200 | 18.0 | 60 | 225 | 5.83 | 1.55 |
| 200 | 30.0 | 60 | 230 | 5.83 | 1.52 |

[a] Size of sample: 350 ml.

[b] No homogenization occurred in this time.

*Procedure C.* Milk was irradiated in the transducer bowl, with the milk serving as the transmission medium. The temperature was controlled to 150° F. ± 5°. The transducer element served as one electrode, and a platinum probe, centrally located, positioned at the focal point of the ultrasonic beam, functioned as the other. A minimum sample volume of approximately 350 ml. was required to fill the bowl. Samples were irradiated with the probe as the positive electrode at different current strengths. These same variations in current strength were repeated with reversed polarities on the electrodes.

Data (Table 3) indicate that an increase in the current through the sample was accompanied by an increase in the homogenization efficiency factor, except when the generator current was 150 M.A., at which level no homogenization was observed. A higher homogenization efficiency was obtained when the platinum probe was the negative electrode.

*Procedure D.* Milk was irradiated directly in the transducer bowl, with the milk as the transmission medium. The temperature was controlled to 150° F. ± 5°. A sintered-glass gas diffusion tube was positioned centrally in the milk-filled transducer bowl, at the focal point of the ultrasonic beam. Samples were irradiated in the presence of percolated nitrogen from a commercial gas cylinder and compared to irradiated controls. The volume of gas passed through the sample was regulated with the pressure-reducer regulator normally available on commercial gas cylinders, and was adjusted to approximately the same quantity, which was evolved electrolytically in the previous Procedures A, B, and C. All samples were treated at a generator output of 200 M.A.

When gas was percolated through the sample, it interfered with the homogenization of the fat in the ultrasonic field. At the conclusion of four hours of ultra-

sonic irradiation, in the presence of percolated gas, no homogenization of the fat was noted; whereas, three hours of irradiation in the absence of percolated gas was sufficient to cause homogenization.

## DISCUSSION

Theoretical considerations of the various phenomena exhibited by ultrasonic energy suggested the following hypothesis: If emulsification in an ultrasonic field depends upon cavitation and cavitation depends upon the presence of dissolved gases, and if ultrasonic irradiation degasses the substance, the extent of emulsification is limited to how far it can proceed prior to the total expulsion of the supporting medium, *i.e.*, dissolved gas.

Two methods for maintaining a required gas content are possible: *(a)* by producing a gas within the substance, through the application of a direct current to electrodes within the substance, or *(b)* by percolating an inert gas through a diffusion tube into the volume of substance being radiated.

The data in Table 1 *(Procedure A)* demonstrated that radiation with ultrasonics, simultaneously with the passage of an electric current through the sample, improved the efficiency of homogenization. Whether the results were owing to the gases generated within the milk, or to the passage of the electric current, is not clearly demonstrated. The experimental observations reported in Table 2 *(Procedure B)* demonstrate the same phenomena. However, in this case there exists a possibility that the polarities of the central electrode and the metallic test tube affect the homogenization efficiency, although the results were indecisive regarding the effect of relative polarity of the container and the central probe. Data from Table 3 *(Procedure C)* support the observation that the passage of an electric current through the sample improves the homogenization efficiency factor, especially when the transducer bowl is the positive electrode and the central probe is negative.

In general, the passage of an electric current through milk while it is being irradiated with ultrasonic energy improved the efficiency of homogenization. Hydrogen ions which naturally exist within the milk are attracted to the negative electrode, where they pick up an electron. Possibly the gas molecules became nuclei for cavitation within the high-intensity radiation region, and contributed to an increased efficiency of homogenization. Homogenization efficiency was not aided when a gas was passed through the milk, possibly because of the presence of large gas cells. These observations lead to the conjecture that although a gas phase is necessary for homogenization to occur, the size of the gas-bubble is an important factor. When the bubbles are too large, they act as cushions, and absorb the energy of cavitation; whereas, when they are submicroscopic in size, as they would be when generated electrolytically, they are beneficial to the phenomenon. The gas nuclei are most effective when they are concentrated in the high-intensity region of the ultrasonic beam.

## SUMMARY

This study was concerned principally with determining the influence of a dissolved gas phase and/or an electric current on the efficiency of homogenization.

There is evidence of a positive relation between homogenization efficiency and current strength through the sample. Theoretically, there should be an optimum electric current strength, and the homogenization efficiency should be expected to increase with an increase in current strength, reach maximum, then decrease, with a further increase in current. Such an experimental relationship was observed. During radiation in a metallic tube, the efficiency factor was not affected conclusively by reversing the polarity of the container and the platinum probe. But, when the milk was radiated directly in the transducer bowl, the homogenization efficiency factor improved when the central electrode was negatively charged.

## CONCLUSIONS

Homogenization by ultrasonic waves was influenced by the passage of an electric current through the milk during radiation.

There was an optimum current strength for maximum homogenization efficiency.

The efficiency of homogenization was dependent upon the relative polarity of the transducer bowl and the electrode, at the focal point in the irradiation zone. The highest homogenization efficiency was obtained when the center electrode was negatively charged.

## REFERENCES

(1) Anderson, E. O. Sonic Vibration of Ice Cream Mixes. *Proc. 36th Ann. Conv., Assoc. Ice Cream Mfgrs.*, 2: 126. 1937. (Cited in *J. Dairy Sci.*, 20: 98. 1937.)

(2) Aston, C. Treating Milk with Sound Waves. *Chem. Abstr.*, 33: 1832. 1939.

(3) Brown, E. P. Homogenization of Milk by Sonic Vibration. *Milk Plant Monthly*, 30: 52, 60. 1941.

(4) Burger, Maria, and Winder, W. C. Homogenization and Deaeration of Milk by Ultrasonic Waves. *J. Dairy Sci.*, 37: 645. 1954.

(5) Chambers, L. A., and Gaines, N. J. Some Effects of Intense Audible Sound on Living Organisms and Cells. J. Cellular Comp. Physiol., 1: 451. 1932.

(6) Elliott, J. A., and Winder, W. C. Effects of Ultrasonic Waves on the Bacterial Flora of Milk. *J. Dairy Sci.*, 38: 598. 1955.

(7) Fox, F. E., and Griffing, V. Experimental Investigation of Ultrasonic Intensity Gain in Water Due to Concave Reflections. *J. Acoust. Soc. Amer.*, 21: 352. 1949.

(8) Harvey, E. M., and Loomis, A. L. The Destruction of Luminous Bacteria by High-Frequency Sound Waves. *J. Bacteriol.*, 17: 373. 1929.

(9) LeGally, D. P., and Patterson, G. W. The Action of High-Frequency Sound Waves on Bacteria. *Am. J. Pharm.*, 112: 373. 1940.

(10) Liu, S. C., and Yen, A. G. N. Further Studies on the Effect of Supersonic Waves on Bacteria. *Proc. Soc. Exptl. Biol. Med.*, 32: 485. 1934.

(11) Loo, C. C., and Carleton, W. M. Further Studies of Cavitation in the Homogenization of Milk Products. *J. Dairy Sci.*, 36: 64. 1953.

(12) Nelson, J. H., and Winder, W. C. Further Studies on the Use of Ultrasonic Waves to Accelerate the Ripening Rate of Cheese. *J. Dairy Sci.*, 37: 638. 1954.

(13) Wood, R. W., and Loomis, A. L. The Physical and Biological Effects of High-Frequency Sound Waves of High Intensity. *Phil. Mag.*, 47: 417. 1927.

# SOME SOURCES OF VARIATION IN TOTAL PLASMA CHOLESTEROL LEVELS IN DAIRY CATTLE [1]

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**Variations in plasma cholesterol levels among animals of varying physiological states and ages were investigated. Effects due to variation among animals were 97.07% of the total variance, while short-term variation in blood sampling was only 2.79%. Plasma cholesterol levels in 11 cows at parturition were significantly lower than either prepartum or postpartum levels. Age, pregnancy, and lactation also affected cholesterol levels.** *Editor.*

The assay of total plasma cholesterol has gradually become recognized as another adjunct in the estimation of thyroid activity, although it is less reliable than more direct measures, such as plasma protein-bound iodine and radioactive-iodine techniques. Numerous workers *(6, 7, 9, 16)* have shown that hyperthyroidism results in hypocholesterolemia; whereas, high levels of total plasma cholesterol are observed in the hypothyroid animal.

The normal level of total plasma cholesterol in dairy cattle has been reported by Boyd *(4)* to be 110 mg. %. Several investigators have studied normal levels of total plasma cholesterol due to age *(20, 22, 24)*, pregnancy and lactation *(2, 14, 18, 20)*, and diet *(17)*. Parturition has been found by Long *et al. (11)* to result in a 20% decrease in plasma concentration of total cholesterol.

Fluctuations in blood levels of total cholesterol have been studied by several workers, in dairy calves *(13)* and humans *(3, 5, 23)*, and have failed to detect significant diurnal or daily variation.

The purpose of this study was to determine the variation in total plasma cholesterol due to differences among dairy animals of varying physiological states, possible daily and diurnal rhythm effects, assay variability, and effects due to parturition. This investigation was not designed to study the specific effects due to different feeding regimens, and it was not possible to remove the effects due to rations, from the effects due to basic differences among groups of animals of varying physiological states.

## EXPERIMENTAL PROCEDURES

The animals used in the first phase of this study were identical to those employed in a previous study on normal variation in plasma PBI levels *(8)*. The four groups of Holstein and Guernsey dairy animals consisted of three pregnant, nonlactating cows; three lactating, nonpregnant cows; three open heifers, 15 to 18 months old, and three heifer calves, 3 to 6 mo. of age. The

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feeding regimens for the pregnant cows consisted of the following poundages per day: grass silage, 30; mixed hay, 10; grain, 6 to 8, and molasses, 3 to 4. The lactating cows received grass silage, 40; mixed hay, 15; grain, 16, and citrus pulp, 8. Proximate analysis of the grain given to both groups indicated 20.14% crude protein, 2.34% crude fat, and 7.65% crude fiber. The 15- to 18-month-old heifers received approximately 30 lb. of grass silage, 10 lb. of mixed hay, and 2 lb. of a fitting ration containing a minimum of 14% crude protein and 3.25% crude fat, and a maximum of 10% crude fiber. The 3- to 6-month-old calves were given mixed hay ad libitum and 4 lb. of calf starter containing not less than 19% crude protein and 3.5% crude fat, and not more than 7% crude fiber.

Three runs were conducted, each run constituting four animals (one animal from each group). Blood samples were collected from each animal at 8 A.M., 4 P.M., and 12 P.M., on each of four alternate days for 1 wk. Duplicate cholesterol determinations were performed on each plasma sample, using the method of Pearson *et al. (12)*. The analysis of variance presented in this phase of the study employed the methods of Snedecor *(21)*. The expectations of mean squares for fixed and random effects were determined by the method of Schultz *(19)*.

In order to study the effect of parturition on plasma levels of total cholesterol, 11 cows (nine Holstein and two Guernsey) were sampled at 2 wk. prepartum, at parturition, and at 2, 4, and 8 wk. postpartum. Duplicate determinations of total plasma cholesterol were performed on each plasma sample. Parturient cows changing from a prepartum to postpartum condition were fed appropriate rations in accordance with their physiological states.

## RESULTS AND DISCUSSION

Mean values for total plasma cholesterol determinations in the first experiment, arranged according to the hour of the blood collection day and the group of animals, are presented (Table 1). Very little difference was found in the mean values for the hour of collection; whereas, considerable difference was noted among the groups of animals. The pregnant, nonlactating group of cows (sampled approximately 2 wk. prepartum) consistently were found to have the highest total cholesterol levels.

An analysis of variance of the data is presented (Table 2). Expectations of mean squares and estimated variances were determined for the various sources

TABLE 1

*Effect of the physiological status and hour of sampling on total plasma cholesterol values*

| Group | Total plasma cholesterol | | | |
|---|---|---|---|---|
| | 8 A.M. | 4 P.M. | 12 P.M. | Mean |
| | *(mg. %)* | *(mg. %)* | *(mg. %)* | *(mg. %)* |
| Pregnant, nonlactating cows | 241.87 | 246.56 | 245.42 | 244.62 |
| Lactating, nonpregnant cows | 96.07 | 95.56 | 95.56 | 95.73 |
| Heifers, 15 to 18 mo. old | 105.63 | 105.59 | 103.95 | 105.06 |
| Calves, 3 to 6 mo. old | 123.60 | 122.69 | 119.26 | 121.85 |
| Means | 141.79 | 142.60 | 141.05 | 141.81 |

of variation (Table 3). The sources of variation were further grouped into three main effects: animal effects, blood sampling effects, and the effects due to assay errors (Table 4).

Animal effects in this study were considered to represent the effects due to group, run, and the interaction between group and run. The group effect, variation in total plasma cholesterol values among the four groups of animals, was highly significant statistically and represented a large part of the animal effect. The variance due to group effect constituted approximately 79% of the total. The run effect, variation in values of different animals of the same group from one run to the next, and the interaction between group and run effects, were found to be highly significant statistically, and contributed 8.2% and 10% of the total variance, respectively. Thus, animal effects in this study collectively represented 97.07% of the total variance. Since the effects due to differences among groups of dairy animals of varying physiological states were confounded

TABLE 2

*Analysis of variance of the total plasma cholesterol data*

| Sources of variation | | Degrees of freedom | Mean square | Error term | F |
|---|---|---|---|---|---|
| | *(symbol)* | | | | |
| Total | | 287 | | | |
| Group | G | 3 | 346,612.86 | GR | 23.81[a] |
| Hour | H | 2 | 57.72 | HR | <1.00 |
| G x H | GH | 6 | 78.38 | GHR | <1.00 |
| Run | R | 2 | 46,792.11 | D (R) | 59.10[a] |
| G x R | GR | 6 | 14,559.22 | GD (R) | 31.47[a] |
| H x R | HR | 4 | 263.50 | HD (R) | 2.24 |
| G x H x R | GHR | 12 | 105.86 | GHD (R) | 1.30 |
| Day | D (R) | 9 | 791.68 | A[GHD (R)] | 95.31[a] |
| G x D (R) | GD (R) | 27 | 462.65 | A[GHD (R)] | 55.70[a] |
| H x D (R) | HD (R) | 18 | 117.80 | A[GHD (R)] | 14.18[a] |
| G x H x D (R) | GHD (R) | 54 | 81.31 | A[GHD (R)] | 9.79[a] |
| Assays in G x H x D (R) | A[GHD (R)] | 144 | 8.31 | | |

[a] Statistically significant at the 1% level ($P<0.01$).

TABLE 3

*Components of mean squares and estimated variances*

| Sources of variation | Expectations of mean squares | Estimated variances | |
|---|---|---|---|
| *(symbol)* | | *($\sigma^2$ or $\theta^2$)* | *(%)* |
| G | $\sigma^2_{A[GHD(R)]} + 6\,\sigma^2_{GD(R)} + 24\,\sigma^2_{GR} + 72\,\theta^2_G$ | 4,611.86 | 78.84 |
| H | $\sigma^2_{A[GHD(R)]} + 8\,\sigma^2_{HD(R)} + 32\,\sigma^2_{HR} + 96\,\theta^2_H$ | −2.14 | −0.04 |
| GH | $\sigma^2_{A[GHD(R)]} + 2\,\sigma^2_{GHD(R)} + 8\,\sigma^2_{GHR} + 24\,\theta^2_{GH}$ | −1.15 | −0.02 |
| R | $\sigma^2_{A[GHD(R)]} + 24\,\sigma^2_{D(R)} + 96\,\sigma^2_R$ | 479.17 | 8.19 |
| GR | $\sigma^2_{A[GHD(R)]} + 6\,\sigma^2_{GD(R)} + 24\,\sigma^2_{GR}$ | 587.36 | 10.04 |
| HR | $\sigma^2_{A[GHD(R)]} + 8\,\sigma^2_{HD(R)} + 32\,\sigma^2_{HR}$ | 4.55 | 0.08 |
| GHR | $\sigma^2_{A[GHD(R)]} + 2\,\sigma^2_{GHD(R)} + 8\,\sigma^2_{GHR}$ | 3.07 | 0.05 |
| D(R) | $\sigma^2_{A[GHD(R)]} + 24\,\sigma^2_{D(R)}$ | 32.64 | 0.56 |
| GD(R) | $\sigma^2_{A[GHD(R)]} + 6\,\sigma^2_{GD(R)}$ | 75.72 | 1.30 |
| HD(R) | $\sigma^2_{A[GHD(R)]} + 8\,\sigma^2_{HD(R)}$ | 13.69 | 0.24 |
| GHD(R) | $\sigma^2_{A[GHD(R)]} + 2\,\sigma^2_{GHD(R)}$ | 36.50 | 0.62 |
| A[GHD(R)] | $\sigma^2_{A[GHD(R)]} +$ | 8.31 | 0.14 |
| Total | | 5,849.58 | 100.00 |

by differences in feeding regimens, no attempt has been made to segregate specific ration effects.

The sum of hour, day (within-run), and the interactions of hour and day effects with each other and with group and run effects, were considered to constitute blood sampling effects and represent short-term variation (within 1 wk.) in levels of total plasma cholesterol levels. The variance due to these combined sampling effects constituted 2.8% of the total variance (Table 4). Daily variation in total plasma cholesterol values was highly significant statistically, yet contributed less than 1% to the total variance. Diurnal variation, as measured by hour effects, was not statistically significant and contributed nothing to the total variance. This confirms other reports *(3, 5, 13, 23)*, which have failed to show significant diurnal variation in total plasma cholesterol levels. Blood samples for total plasma cholesterol determination may be collected at any time during the day.

TABLE 4

*Main sources of variation in total plasma cholesterol values*

| Combined sources of variance | Estimated variances | |
|---|---|---|
| | *($\sigma^2$ or $\theta^2$)* | *(%)* |
| Animal | 5,678.39 | 97.07 |
| Group | 4611.86 | 78.84 |
| Run | 479.17 | 8.19 |
| G x R | 587.36 | 10.04 |
| Sampling—S[a] | 162.89 | 2.79 |
| Assay—A[GHD(R)] | 8.31 | 0.14 |
| Total | 5,849.59 | 100.00 |

[a] Consists of H, D(R), HD(R), and interactions of H and D(R) with G and R.

The variance due to assay errors in total plasma cholesterol determinations, represented by variation among duplicate determinations, accounted for only 0.14% of the total variance. Little would be gained by performing more than one determination per blood sample.

The standard error of a single cholesterol determination, derived from the error term variances for sampling and assay error effects, was found to be 10.69 mg. %. The coefficient of variation was 7.53%. It can be seen (Table 5) that a greater reduction in the standard error and coefficient of variation is possible by the use of more blood samplings, rather than by the use of more determinations per blood sample. However, since the variance due to the difference among animals represents most of the total variance (97.07%), it does not appear feasible to collect more than one blood sample per animal for routine determinations of total plasma cholesterol.

Results of the effect of parturition on cholesterol values in 11 dairy cows are presented (Table 6). In all instances, the plasma level of total cholesterol was significantly lower at parturition than at any other time during the sampling period. This finding is in good agreement with that of Long *et al. (11)*. The marked effect of parturition on cholesterol level is not believed to be attributable to slight quantitative differences in rations fed to pre- and postpartum cows. It is interesting to note that the level of plasma PBI also decreases at parturition

TABLE 5

*Effect of varying the number of blood samples and the number of assay determinations per sample, on the standard error and coefficient of variation of total plasma cholesterol*

| No. of determinations | No. of blood samples | | | | | |
|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | |
| | S.E.[a] | C.V.[b] | S.E. | C.V. | S.E. | C.V. |
| | *(mg. %)* | *(%)* | *(mg. %)* | *(%)* | *(mg. %)* | *(%)* |
| 1 | 10.69 | 7.53 | 7.56 | 5.32 | 6.17 | 4.34 |
| 2 | 10.49 | 7.39 | 7.42 | 5.22 | 6.06 | 4.26 |
| 3 | 10.42 | 7.34 | 7.37 | 5.19 | 6.02 | 4.24 |

Based on $\sigma^2_S = 162.89$ and $\sigma^2_{A(GHD(E))} = 8.31$.
[a] S.E. = Standard error of a mean cholesterol determination.
[b] C.V. = Coefficient of variation of a mean cholesterol determination.

TABLE 6

*Effect of parturition on levels of total plasma cholesterol (mean of 11 animals)*

| Time | Mean total plasma cholesterol |
|---|---|
| | *(mg. %)* |
| 2 wk. prepartum | 104.57 |
| Partum | 85.92 |
| 2 wk. postpartum | 135.27 |
| 4 wk. postpartum | 193.95 |
| 8 wk. postpartum | 223.01 |

*(10, 15)*. Low levels of total plasma cholesterol, coincident with low plasma PBI values at parturition, may indicate that these levels are not true reflections of thyroid activity.

## SUMMARY

Variation in total plasma cholesterol levels was studied in four groups of dairy cattle of various ages and physiological states, in which three blood samples were collected from each of 12 animals at designated intervals during the day, on each of four alternate days. The effects due to variation among animals constituted 97.07% of the total variance. Short-term variation in blood sampling, represented by daily and diurnal rhythm effects, constituted 2.79% of the total variance; whereas, assay error effects contributed only 0.14% of the total variance.

The physiological state of the animal as expressed by age, parturition, pregnancy, and lactation, had a profound influence on total plasma cholesterol levels. Daily variation in total plasma cholesterol values was highly significant statistically, yet contributed less than 1% to the total variance. No significant diurnal rhythm effect was noted, indicating that blood samples for total plasma cholesterol assay could be collected at any time during the day. One cholesterol determination per blood sample and one blood sample per animal were found to be sufficient for routine investigations.

Levels of total plasma cholesterol in 11 cows at the time of parturition were significantly lower than prepartum or postpartum levels.

## REFERENCES


(1) Barker, H. B., and Jacobson, N. L. Diurnal Variation in Concentrations of Fat in the Blood Plasma of Calves Fed Various Types of Oils. *J. Dairy Sci.*, **32**: 709. 1949.

(2) Boyd, E. M. The Lipemia of Pregnancy. *J. Clin. Invest.*, **13**: 347. 1934.

(3) Boyd, E. M. Diurnal Variations in Plasma Lipids. *J. Biol. Chem.*, **110**: 61. 1935.

(4) Boyd, E. M. Species Variation in Normal Plasma Lipids Estimated by Oxidative Micromethods. *J. Dairy Sci.*, **25**: 131. 1942.

(5) Bruger, M., and Somach, I. The Diurnal Variations of the Cholesterol Content of the Blood. *J. Biol. Chem.*, **97**: 23. 1932.

(6) Byers, S. O. Friedman, M., and Rosenman, R. H. Review: On the Regulation of Blood Cholesterol. *Metabolism*, **1**: 479. 1952.

(7) Hurxthal, L. M., and Simpson, H. N. Hypothyroidism. Hypercholesterolemia. *J. Clin. Endocrinol.*, **1**: 450. 1941.

(8) Lennon, H. D., Jr., and Mixner, J. P. Variation in Plasma Protein-Bound Iodine Levels in Dairy Cattle Due to Animal, Daily, Diurnal, and Method Effects. *J. Dairy Sci.*, **40**: 541. 1957.

(9) Leroy, G. V., Bergenstal, D. M., and Kabara, J. J. The Rate of Cholesterol Synthesis in Patients with Myxedema. *J. Lab. Clin. Med.*, **44**: 827. 1954.

(10) Lewis, R. C. Protein-Bound Iodine Levels in Dairy Cattle Plasma, Milk and Colostrum. Unpublished Ph.D. thesis. Michigan State College of Agriculture and Applied Science. 1952.

(11) Long, J. F., Hibbs, J. W., and Gilmore, L. O. The Effect of Thyroprotein Feeding on the Blood Level of Inorganic Iodine, PBI, and Cholesterol in Dairy Cows. *J. Dairy Sci.*, **36**: 1049. 1953.

(12) Pearson, S., Stern, S., and McGavack, T. H. A Rapid, Accurate Method for the Determination of Total Cholesterol in Serum. *Anal. Chem.*, **25**: 813. 1953.

(13) Ramsey, H. A., Wise, G. H., Tove, S. B., and Waugh, R. K. Effects of Various Diets on the Diurnal Patterns of Blood Plasma Lipids of Dairy Calves. *J. Dairy Sci.*, **37**: 1357. 1954.

(14) Reder, R. The Lipase, Fatty Acid and Cholesterol Content of Cow's Blood in Relation to the Production of Rancid Milk. *J. Dairy Sci.*, **21**: 475. 1938.

(15) Robertson, W. G., Lennon, H. D., Jr., Bailey, W. W., and Mixner, J. P. Interrelationships Among Plasma 17-Hydroxycorticosteroid Levels, Plasma Protein-Bound Iodine Levels, and Ketosis in Dairy Cattle. *J. Dairy Sci.*, **40**: 732. 1957.

(16) Rosenman, R. H., Friedman, M., and Byers, S. O. The Mechanism Responsible for the Altered Blood Cholesterol Content in Deranged Thyroid States. *J. Clin. Endocrinol. and Metabolism*, **12**: 1287. 1952.

(17) Saarinen, P., and Shaw, J. C. Studies on Ketosis in Dairy Cattle. XII. Blood Lipids, Phosphates and Phosphatase Activity of Cows on Different Levels of Feed Intake Postpartum. *J. Dairy Sci.*, **33**: 508. 1950.

(18) Schaible, P. J. Plasma Lipids in Lactating and Nonlactating Animals. *J. Biol. Chem.*, **95**: 79. 1932.

(19) Schultz, B. F., Jr. Rules of Thumb for Determining Expectations of Mean Squares in Analysis of Variance. *Biometrics*, **2**: 123. 1955.

(20) Shope, R. E. The Effect of Age on the Total and Combined Cholesterol of the Blood Serum. *J. Biol. Chem.*, **80**: 141. 1928.

(21) Snedecor, G. W. *Statistical Methods.* 5th ed. Iowa State College Press, Ames. 1956.

(22) Teeri, A. E., Keener, H. A., and Morrow, K. S. Studies on the Chemical Composition of Calf Blood. *J. Dairy Sci.*, **29**: 663. 1946.

(23) Turner, K. B., and Steiner, A. A Long-Term Study of the Variation of Serum Cholesterol in Man. *J. Clin. Invest.*, **18**: 45. 1939.

(24) Zaletal, J. H., Allen, R. S., and Jacobson, N. L. Lipids in Blood Plasma of Young Dairy Calves. *J. Dairy Sci.*, **35**: 1046. 1952.

# TWINNING IN DAIRY CATTLE AND ITS RELATION TO PRODUCTION[1]

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**Twinning and its effects on fat production were investigated in the Iowa State College dairy herd and in nine herds of the Iowa Board of Control. Twin births were 2.9 per cent of the total of 10,885 births. From the sex ratio, the frequency of identical twins was estimated as 8.6 ± 1.1 per cent. In lifetime average production per 10-month lactation, the dams of twins averaged 19 ± 9 lb. of butterfat higher than their herd mates, but this was almost wholly due to confounding the effects of selection with the effects of age on twinning frequency. The difference fell to 3 ± 6 lb. when dams of twins were compared only with contemporary herd-mates who remained in the herd equally long. Production in the lactation following the birth of twins was 21 ± 11 lb. lower than in the preceding lactation.** *Editor.*

This investigation was prompted by occasional inquiries from dairymen concerning the frequency of twins among dairy cattle, and by remarks that high-producing cows are more likely to have twins, but that their production following the birth of twins is lower.

Investigations of twin frequencies among dairy cattle *(2, 12)* indicate differences between breeds. Frequencies obtained from herd records appear to be slightly higher than those obtained from the herd-books kept by breed societies.

Several authors have compared the milk and butterfat production of dams of twins with the production of other members of the herd, or with the herd average (*8, 10, 18, 19, 22*). In every case, the average production of dams of twins was higher than that of cows which produced only singles. For example, Pfau *et al. (22)* reported the average daily milk production of 26 dams of twins to be 30.1 ± 1.1; whereas, the herd average was 29.1 ± 0.6. This difference was not statistically significant ($P = 0.70$), but the consistency with which such differences are found is impressive.

Frequency of twins increases with age of dam, at least up until maturity (*8, 12, 13, 22*). This is important in comparing dams of twins with other cows, since most dams of twins will have stayed in the herd for several lactations.

Wilson (*25*) compared the lactations following twin births with those before and after such births. Production in the lactation following a twin birth was less by 1,000 lb. of milk and 50 lb. of butterfat.

Data for the present study came from the Iowa State College herd and nine herds of the Iowa Board of Control; all of the cows in the latter were purebred Holstein-Friesians. Accurate birth records are maintained for all of these herds. Abortions, stillbirths, and normal births were all included, provided the herds-

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man recorded the sex. All these herds were on DHIA or HIR production testing during the period studied. All production records, 30 days or longer, were converted to a 305-day 2× mature equivalent basis *(15)*. Only Holstein-Friesian production records from 1940–1952 inclusive were used, but the data on frequency of twins in the college herd include all birth records of all breeds from 1910–1952, inclusive. Such culling as was practiced in these herds was mostly based on first and second lactation records, but no selection for or against twinning was done, so far as is known.

## RESULTS

Of the 10,885 births examined, 317 were twin births. This information is shown near the bottom of Table 1, along with other data taken from the literature, which seem to have been based on herd records rather than on studies of registrations. The 317 twin births here showed a sex combination ratio of: 91 ♂♂ : 151 ♂♀ : 75 ♀♀.

TABLE 1

*Twin births among dairy cattle in the United States*

| Author | Breed | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ayrshire | | Guernsey | | Holstein | | Jersey | | Brown Swiss | | Total | |
| | All | Twins | All | Twins | All | Twins | All | Twins | All | Twins | All | Twins |
| *(6)* | | | | | | | | | | | 2,573 | 21 |
| *(11)* | | | | | 1,986 | 50 | | | | | 1,986 | 50 |
| *(19)* | 123 | 2 | 108 | 3 | | | 97 | 1 | | | 328 | 6 |
| *(16)* | | | | | 2,910 | 86 | | | | | 2,910 | 86 |
| *(17)* | 68 | 3 | 165 | 2 | 466 | 20 | 175 | 3 | 66 | 0 | 940 | 28 |
| *(20)* | | | | | | | | | | | 689 | 21 |
| *(23)* | | | | | 1,954 | 71 | | | | | 1,954 | 71 |
| *(9)* | | | 1,063 | 21 | | | | | | | 1,063 | 21 |
| *(22)* | | | | | 937 | 36 | | | | | 937 | 36 |
| *(21)* | | | 1,165 | 16 | 259 | 13 | 137 | 1 | | | 1,561 | 30 |
| *(4)* | | | | | | | | | | | 1,256 | 37 |
| *(5)* | 364 | 16 | 364 | 13 | 977 | 30 | 657 | 7 | | | 2,362 | 66 |
| *(1)* | | | | | | | 912 | 18 | | | 912 | 18 |
| *(7)* | | | | | | | 536 | 6 | | | 2,607 | 41 |
| Meadows | 334 | 4 | 461 | 10 | 9,247 | 271 | 604 | 5 | 239 | 27 | 10,885 | 317 |
| Total | 889 | 25 | 3,326 | 65 | 18,736 | 577 | 3,118 | 41 | 305 | 27 | 32,963 | 849 |
| Per cent | 2.81 | | 1.95 | | 3.08 | | 1.31 | | 8.85 | | 2.58 | |

Two formulas have been derived for estimating the frequency of identical twins. Weinberg's formula *(24)* assumes the number of identical twins to be the excess of like-sexed twins over unlike-sexed twins. Bonnier *(3)*, using the method of maximum-likelihood, derived another formula for estimating, from the sex ratios actually observed in the sample, the frequency *(m)* of identical twins among like-sexed twins and the variance of this estimate. Naturally, the two formulas give the same results, when the sample sex ratio is exactly 50:50, and they differ little unless the ratio is far from that.[2]

[2] Such difference as there is arises from Bonnier accepting the sample sex ratio as a starting point; whereas, Weinberg assumes implicitly that the sex ratio is 50:50. Note that Bonnier's *m* expresses the identical twins as a fraction of the like-sexed twins, whereas Weinberg's *P* expresses them as a fraction of all twins.

Bonnier's formula is:

$$m = \frac{2npq - n_2}{2pq(n - n_2)} \pm \frac{4(1-m)}{n}(1 - 2mpg)^2$$

$n$ = total pairs of twins

$n_2$ = number of pairs, which are 1 male and 1 female

$p$ = proportion of males

$q = 1 - p$ = proportion of females

In this notation, Weinberg's formula, where $P$ = fraction of total pairs expected to be identical, is: $P = \frac{n-2n_2}{n}$. When $P$ is expressed in terms of $m$, Weinberg's formula becomes: $m = \frac{n - 2n_2}{n - n_2}$.

In the present data, the sex ratio is 525 : 475, and Bonnier's formula estimates the frequency of identical twin pairs among like-sexed pairs to be $8.6 \pm 1.1$. Weinberg's formula would give 15 pairs of identical twins, which is a frequency of 4.7% of the total pairs, or 9.0% of the like-sexed twins. These estimates agree fairly well with those in the literature.

Table 2 shows the average lifetime butterfat production of dams of twins and their contemporary herd-mates, a contemporary being a cow born in the same herd and during the same calendar year as the dam of twins.

To compare the two groups of dams (Table 2), the unequal number of dams in each classification must be considered, since the herds did differ significantly in their levels of production. The method of fitting constants can be used to do an analysis of variance of a two-way classification with disproportionate frequencies. Kempthorne [*(14)*, section 6.3] shows a numerical example worked with no interaction present. From what is known of the management of the herds in this study, dams of twins are not likely to have received better treatment—relative to dams of singles—in one herd than in another. Examination of the means (Table 2) also indicates that if there is any interaction, it is small.

TABLE 2

*Average lifetime butterfat production of dams of twins and production of contemporary herd-mates*

| Herds | Contemporaries | | Dams of twins | | Twin minus contemporary |
|---|---|---|---|---|---|
| | No. cows | Mean | No. cows | Mean | |
| | | *(lb.)* | | *(lb.)* | *(lb.)* |
| 1 | 157 | 409 | 12 | 435 | 26 |
| 2 | 124 | 362 | 25 | 378 | 16 |
| 3 | 161 | 346 | 16 | 376 | 30 |
| 4 | 69 | 389 | 9 | 453 | 64 |
| 5 | 137 | 333 | 18 | 432 | 99 |
| 6 | 274 | 384 | 37 | 384 | 0 |
| 7 | 227 | 400 | 21 | 417 | 17 |
| 8 | 103 | 423 | 7 | 418 | –5 |
| 9 | 77 | 408 | 7 | 451 | 43 |
| 10 | 129 | 376 | 18 | 406 | 30 |
| Total | 1,458 | 390 | 170 | 407 | 17 |

However, a component of variance analysis was done (shown Table 3). This analysis did not show a significant interaction. Hence, the interaction was considered to be negligible.

The butterfat production of dams of twins exceeded that of contemporary dams of singles by 19.3 ± 8.9 lb. Although this difference is more than twice as large as its standard error, two factors must be considered that might influence the results. First, the lifetime averages were computed from a varying number of records. The incomplete repeatability of production records must be considered, if this comparison is to be completely valid. Second, the record following the birth of twins is included in the lifetime average of dams of twins. If these affect the record seriously, the difference would be somewhat larger.

Johansson *(12)* reported that the total birth-weight of twins exceeded that of single calves by 56.6% among Swedish Red and White cattle, and by 36.0% among Swedish Friesians. The metabolic strain of producing the excess weight of twin calves might have influenced the lactation following twin births.

TABLE 3

*Analysis of variance to evaluate the interaction component of variance*

| Source | d.f. | Mean square | Expected composition of mean square |
|---|---|---|---|
| Total | 1,627 | 6,059 | |
| Herds | 9 | 88,196** | E + 130.1 GH + 0.3G + 159.7 H |
| Groups (*i.e.*, twin-producers *vs.* others) | 1 | 38,237** | E + 37.1 GH + 301.8G + 1.3 H |
| Herd × groups | 9 | 6,357 | E + 29.4 GH − 0.3G − 0.1 H |
| Error | 1,608 | 5,327 | E |
| Components:– Herds 487.9 — Groups 102.5 — Group × herds 37.7 | | | |

[a] $P < 0.01$ if the insignificant GH is considered zero.

The difference between two records made by the same cow includes the effect of age and of any change in environment from one lactation to the next. Age-conversion tables are in use and, on the average, give reliable results. Environmental variations are important, although difficult to measure. Intra-herd correlations between age-corrected records of the same cow are about 0.40. If a control cow could be found of the same age, in the same herd, and with two lactations beginning at about the same time as the record preceding the birth of twins and the lactation following, then comparing the change in size of records should give an estimate of the effect of twinning. This estimate would include the effects of any changes in environment which were not the same for the control cow and for the dam of twins. Age conversion would not be a factor and any general changes in environment which affected alike all the cows in the herd at that time would be eliminated. This general idea of selecting for each "propositus" or "proband" or "index case" a control matched to eliminate nonpertinent variables, is widely used in research in human genetics, where controlled experiments in the laboratory sense are not possible.

Accordingly, a control cow for each dam of twins was selected from among its contemporary herd-mates. The contemporary cow whose birth-date was nearest to that of the dam of twins was selected from among those having two records

beginning at nearly the same time as those of the dam of twins. Means of these four records are shown (Table 4). That the 375 is lower than the other three averages suggests that the birth of twins causes the ensuing lactation to yield about 5% less than if the birth had been a single. An analysis of variance was done (shown in Table 5). The lactation record following the birth of twins was $21.0 \pm 11.0$ lb. of butterfat lower than the preceding record. This approaches statistical significance ($13{,}563 \div 3967 = 3.42$) at the 0.05 level.

The comparison of lifetime production of dams of twins with contemporary herd-mates (Table 2) had failed to allow for any selection in the herds. Selec-

TABLE 4

*Mean production of dams of twins and of their controls*

| Dams of Twins | | Controls | |
|---|---|---|---|
| $T_1$ | 375 | $S_1$ | 402 |
| $T_2$ | 400 | $S_2$ | 406 |

($T_1$)—record following the birth of twins; ($T_2$)—same cow's record preceding the birth of twins; ($S_1$)—record of a control cow beginning at or about the same time as the $T_1$ record; ($S_2$)—record of control cow beginning at or about the same time as the $T_2$ record.

tion practiced in the herds from which these data were taken is based for the most part on first and second production records, as is generally true in most dairy herds. Twinning increases with age of dam, and those cows permitted to remain in the herd through maturity, have a much better chance of producing twins. Hence, what appears in Table 2 to be superior producing-ability of dams of twins may be only an incidental result of the dams of twins being a more selected group, since the longer a cow stayed in the herd the more chance it had of becoming a dam of twins.

To test this more definitely, each dam of twins was paired with a contemporary herd-mate, which remained in the herd for the same number of lactations. Since the dam of twins and its control cow remained in the herd the same period of time, the herdsman had not expressed in his actions any preference between these two cows. The record made following the birth of twins was eliminated

TABLE 5

*Analysis of variance for effect of twin births*

| Source | d.f. | Mean square | Expected composition of mean square |
|---|---|---|---|
| Total | 527 | 6,994 | |
| Among pairs | 131 | 12,754 | |
| Type of record | 3 | | |
| $(S_1 + T_1) - (S_2 + T_2)$ | 1 | 37,538 | E + A + 132J + 264Y |
| $(S_1 + S_2) - (T_1 + T_2)$ | 1 | 28,131 | E + A + 132J + 264C |
| $(S_1 + T_2) - (T_1 + S_2)$ | 1 | 13,563 | E + A + 132J |
| Remainder | 393 | | |
| Pair × year | 131 | 6,849 | E + A + 2D |
| Pair × cow | 131 | 3,961 | E + A + 2B |
| Pair × cow × year | 131 | 3,967 | E + A |
| F = 13563/3967 = 3.42 | | | |

E, error; Y, year; C, cow; B, pair × cow; D, pair × year; J, year × cow; A, pair × year × cow.

from the lifetime production record of the dam of twins. The control animal's record starting at about the same time was also removed. In every case in which two or more contemporary cows met the requirements for a control, the cow whose birth-date was nearest that of the dam of twins was chosen.

The means of these two groups were 406 lb. of butterfat for dams of twins, and 403 for controls. The analysis of variance for comparing these two groups is shown (Table 6). The mean square for the "remainder" is actually larger

TABLE 6

*Analysis of variance in production of dams of twins and of herd-mates remaining in the herd the same number of lactations*

| Source | d.f. | Mean square | F |
|---|---|---|---|
| Total | 339 | 3,942 | |
| Between pairs | 169 | 5,288 | |
| Between dams of twins and controls | 1 | 789 | 0.31 |
| Remainder | 169 | 2,615 | |

than that "between dams of twins and controls," although not significantly so. This is probably only a sampling variation because, in selecting the controls, no attention was paid to the size of their records. We conclude that any difference in intrinsic productiveness, as between dams of twins and cows which produce only singles, is still unproven. If any such difference does exist, it must certainly be too small to deserve any attention in practice.

## SUMMARY

In these data, twin births were 2.91% of the total births. The frequency of identical twins among like-sexed pairs was estimated to be $8.6 \pm 1.1\%$.

The average lifetime production of dams of twins was $19.3 \pm 8.9$ lb. of butterfat higher than the average production of all contemporary herd-mates. Because frequency of twinning increases with age of dam, this comparison is biased by early culling and selection. Cows removed from the herd after one or two records do not have as much chance to become dams of twins as those cows which are kept for many lactations. When dams of twins were compared with contemporary herd-mates which remained in the herd equally long, the difference was only $3.0 \pm 5.6$ lb.

The butterfat production in the lactation following the birth of twins was $21.0 \pm 11.0$ lb. lower than in the lactation immediately preceding this birth. This we consider an estimate of the amount by which the production following the birth of twins is lowered by the physiological extra load, or strain, of producing twins.

## REFERENCES

(1) Arnold, P. T. Dix, and Becker, R. B. Dairy Calves, Their Development and Survival. Fla. Agr. Expt. Sta., *Bull.* 529. 1953.

(2) Belic, Milan. Zwillings- und Drillingsuntersuchungen beim Rind unter besonderer Berücksichtigung der Skelettvariabilität. *Z. Tierzücht. Züchtungsbiol.*, **49**: 10. 1941.

(3) Bonnier, Gert. Studies on Monozygous Cattle Twins. II. Frequency of Monozygous Twins. *Acta Agr. Suecana, I*, (**2**): 147. 1946.

(4) Brakel, W. J., Rife, D. C., and Salisbury, S. M. Factors Associated with the Duration of Gestation in Dairy Cattle. *J. Dairy Sci.*, **35**: 179. 1952.
(5) Davis, H. P. Dairy Calf Birth and Disposals. Nebr. Agr. Expt. Sta., *Bull.* 411. 1952.
(6) Gowen, John W. Report of Progress on Animal Husbandry Investigations in 1917. Maine Agr. Expt. Sta., *Bull.* 274. 1918.
(7) Herman, H. A., Spalding, R. W., and Bower, K. W. Factors Affecting Length of Gestation Period in Dairy Cattle. Univ. of Missouri, *Research Bull.* 592. 1953.
(8) Hewitt, A. C. T. Twinning in Cattle. *J. Dairy Research*, **5**: 101. 1934.
(9) Calving Periods. *Hoard's Dairyman*, **90**: 333. 1945.
(10) Hunt, G. A. Relation of Milk Production to the Twinning Tendency. *J. Dairy Sci.*, **7**: 263. 1924.
(11) Jewell, F. M. Sex Ratio in Foetal Cattle. *Biol. Bull.*, **41**: 257. 1921.
(12) Johansson, Ivar. The Sex Ratio and Multiple Births in Cattle. *Z. Tierzücht. Züchtungsbiol.*, **24**: 165. 1932.
(13) Jones, S. V. H., and Rouse, H. E. Multiple Births in Cattle and Sheep. *J. Dairy Sci.*, **3**: 260. 1920.
(14) Kempthorne, Oscar. *The Design and Analysis of Experiments.* John Wiley and Sons, N.Y. 1952.
(15) Kendrick, J. E. Standardizing DHIA Records in Proving Sires. USDA, Bur. Dairy Ind., BDI-INF 162. 1953.
(16) Knott, J. C. A Study of Gestation Period of Holstein-Friesian Cows. *J. Dairy Sci.*, **15**: 87. 1932.
(17) Lamb, Leland W. Multiple Births in Dairy Cattle. Mich. Agr. Expt. Sta., *Quart. Bull.*, **17**: 185. 1935.
(18) Ljutikov, K. M. Twinning in the Bestuzhev Breed of Cattle. *Véstnik. Ziovotn.*, **4**: 21. 1946. (Original not available for examination; abstracted in *Animal Breeding Abstr.*, **15**: 107. 1947.)
(19) Lush, Robert H. Inheritance of Twinning in a Herd of Holstein Cattle. *J. Heredity*, **16**: 273. 1925.
(20) Miller, Fred W., Graves, R. R., and Fohrman, M. H. Management and Breeding Data on a Dairy Herd in which Bang's Disease (Infectious Abortion) was Eradicated by Segregation. *J. Dairy Sci.*, **20**: 537. 1937.
(21) Miller, K., and Gilmore, L. Calf Mortality, Sex Ratio, and Incidence of Twinning in Two University of Minnesota Herds. *J. Dairy Sci.*, **32**: 706. 1949.
(22) Pfau, K. O., Bartlett, J. W., and Shuart, C. E. A Study of Multiple Births in a Holstein-Friesian Herd. *J. Dairy Sci.*, **31**: 241. 1948.
(23) Porter, Arthur R. A Study of Breeding Records in Minnesota Institution Herds. Univ. of Minnesota. (Unpublished research.) 1939.
(24) Weinberg, W. Beiträge Zur Physiologie und Pathologie der Mehrlingsgeburten beim Menschen. *Pflügers Arch. ges. Physiol.*, **88**: 346. 1902.
(25) Wilson, Chase. Effects of Twinning in Dairy Cattle. *S. Dakota Farm and Home Research*, **6**: 107. 1955.

# A STUDY OF IRON AND COPPER REQUIREMENTS OF DAIRY CALVES[1,2]

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**The dietary requirements of dairy calves for iron and copper were investigated, using 27 male Holstein calves. Calves receiving no supplemental iron became anemic and gained less than those on 30 mg. and 60 mg. intakes of iron, daily. Thirty or 60 mg. of iron daily was sufficient either to maintain normal red-cell volumes and hemoglobin or to increase the hemoglobin of anemic calves from an average of 5.4 g. to 8.0 g. per 100 ml. during 16-20 weeks. There was no difference in hemoglobin between the 30 and 60 mg. intake levels, but 60% of the 30 mg. and 30% of the 60 mg. level of iron were utilized. Without supplemental copper, serum copper decreased, but an intake of 6 mg. of copper, daily, maintained it at a normal level.** *Editor.*

Although iron and copper apparently are essential for all animals thus far studied, the quantitative requirements of these metals have been determined for only a few species. Definitive information about the requirements of these metals for dairy calves is lacking.

Knoop, Krauss, and Washburn *(22)* reported that calves on an exclusive milk diet developed anemia at about 120 days of age, as evidenced by unthriftiness, retarded growth, and low hemoglobin concentration. The daily (except Sundays and holidays) addition of 400 mg. of inorganic iron and 40 mg. of inorganic copper to the milk diet prevented the development of nutritional anemia in calves. Thomas *et al. (30)* reported that the feeding of 100 mg. of iron daily to calves that were moderately anemic caused an increase in the number of their red blood cells and hemoglobin levels. No attempt was made to estimate minimum dietary requirements in either investigation.

Bennetts *et al. (3)* reported that the daily intake of 60 mg. of copper prevented anemia in grazing cattle in Western Australia and maintained normal hemoglobin and copper concentrations in blood. Becker *et al. (2)* recommended a mineral mixture containing 1% copper sulfate for grazing cattle in copper-deficient areas. Deficiency symptoms did not develop if the animals consumed as much as ½ oz. of this mixture per day. Mitchell *(24)* estimated that 5 p.p.m. of copper in the diet were sufficient for the growing calf, an amount 10- to 20-fold less than that recommended by other investigators. It is possible that the anemic condition which Bennetts *et al. (3)* and Becker *et al. (2)* attributed to a lack of copper was a complicated deficiency caused by an excess of other minerals *(9, 11)*.

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[2] Paper is based, in part, on data presented in a thesis submitted by C.C. in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the animal nutrition field.

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In view of the exiguous data on the dietary requirements of dairy calves for iron and copper, a study of the requirements and possible interrelationships of these metals in the nutrition of calves was undertaken.

## EXPERIMENTAL PROCEDURE

Twenty-seven male Holstein calves obtained at one to three days of age were used in this study. The basal diet was whole milk, supplemented daily with 10,000 U.S.P. units vitamin A, 1,000 U.S.P. units vitamin D,[4] 0.6 g. magnesium, and 0.1 mg. cobalt, per 100 lb. of body weight.

To obtain information on requirements per se and on possible interrelationships between iron and copper, three levels of iron and three levels of copper were studied in a 3 × 3 factorial design *(7)*, involving a total of nine treatments as follows:

| Levels of copper | Levels of iron | | |
|---|---|---|---|
| | *0* | *30* | *60* |
| | *(mg/day)* | | |
| 0 | 0:0 | 0:30 | 0:60 |
| 3 | 3:0 | 3:30 | 3:60 |
| 6 | 6:0 | 6:30 | 6:60 |

The investigation was conducted in two trials, 1 and 2. In the first, nine calves were started on the experimental treatments at approximately two days of age. Because of possible confounding effects arising from variable stores of iron and copper in the tissues, a depletion procedure similar to that reported by Mitchell and Miller *(25)* was used in the second trial. Eighteen calves were fed the basal diet for a preliminary period, beginning at approximately two days of age and continuing for each animal until its hemoglobin level declined to 5-6 g. per 100 ml. of blood, at which time the anemic calf was assigned at random to one of the experimental treatments.

The calves were placed into individual pens bedded with wood shavings. Stainless steel muzzles were kept on the calves at all times, except at feeding, to minimize the intake of extraneous materials. The milk, which was collected and stored in stainless steel containers, was fed from nippled pails at the rate of 10 lb. per 100 lb. body weight. Samples of milk were collected periodically, at feeding time, and analyzed for iron and copper.

The dietary supplemental iron [5] used in this experiment was dissolved in hydrochloric acid and diluted with redistilled water to contain 15 mg. of iron per ml. of solution; the copper [6] was dissolved in hydrochloric and nitric acids and diluted with redistilled water, to the extent that the final solution contained

[4] Vitamins A and D contributed by the R. P. Scherer Corporation, Detroit 13, Michigan.

[5] Carbonyl iron powder, grade $R_x$, obtained from General Aniline and Film Corporation, 444 Madison Avenue, New York 22, New York.

[6] Copper metal, 0.008-inch thick, electrolytic sheet, J. T. Baker Chemical Company, Phillipsburg, New Jersey.

3 mg. of copper per ml. The resulting copper solution was found to be free of iron, and, likewise, the iron solution was free of copper.

The daily doses of iron and copper were diluted with about 50 ml. redistilled water and given by drenching, from 1 to 3 hr. prior to the afternoon feeding. This schedule was followed to minimize the possible formation of complexes with milk constituents such as calcium, phosphorus, and proteins *(4, 19, 21, 31)*.

The response criteria were: *(a)* general appearance, *(b)* weekly weights, *(c)* hemoglobin concentration in blood, *(d)* serum-iron and -copper concentrations, and *(e)* cell volume.

Blood samples were collected from the jugular vein of each animal at weekly intervals in Trial 1 and at biweekly intervals in Trial 2. The collection time in relation to feeding was adhered to rather closely to reduce the influence of diurnal variations.

The method of Kitzes, Elvehjem, and Schuette *(20)* was used to prepare the serum filtrate for iron analysis, but O-phenanthroline was used instead of $\alpha$, $\alpha$-dipyridyl as a color reagent, Parks *et al. (26)*. The Parks *et al. (26)* method, as modified by Matrone *et al. (23)*, was used for the determination of copper. Hemoglobin was determined by the method reported by Shenk, Hall, and King *(28)*, and cell volume by use of the Sanford Magrath hematocrit tube.

All reagents were purified by distillation in a Pyrex-glass apparatus. Reagent and sample blanks were included with each set of samples. All glassware was washed in a potassium dichromate cleaning solution, rinsed several times in tap water, and then rinsed at least three times with distilled water.

## RESULTS

*General appearance and health.* Although the calves were normal in general appearance, scours was a common disorder, irrespective of dietary treatment. The frequency and severity of scours increased when the calves were about 4 or 5 mo. of age. At this stage, all calves were also irregular in their intake of milk. Other workers *(16, 22)* have reported similar results with calves fed whole-milk diets.

Calves receiving diets without supplemental iron and having low hemoglobin concentrations in the blood tired easily and became dyspneic upon physical exertion. Neither graying of hair *(17, 29)* nor symptoms of bone abnormalities *(9)* were observed among the calves receiving diets without supplemental copper.

Calves that were autopsied had few shavings and hair-balls in the gastrointestinal tract, indicating that the stainless steel muzzles successfully obstructed the intake of foreign material.

*Weight gains.* Although, compared to the Ragsdale *(27)* standards, all calves made at least normal weight gains, the animals fed the basal diet unsupplemented with iron made significantly ($P \leqslant .05$) lower average daily gains than those supplemented with either 30 or 60 mg. iron per day (Table 1). Differences in average daily gains were not significant ($P \geqslant .05$), either in the comparison

between the groups fed the 30- and the 60-mg. levels of iron or in the comparison among the 0, 3, and 6 mg. of copper (Table 1).

*Hemoglobin.* In Trial 1, calves on the 0 level of supplemental iron became anemic at an average rate of 0.779 g. hemoglobin per 100 ml. blood per 2-wk. period; whereas, those receiving either 30 or 60 mg. of supplemental iron per day maintained a normal level of hemoglobin, 10 to 13 g. per 100 ml. blood, throughout the 36- to 40-wk. experimental period (Figure 1). Differences between hemoglobin levels of the calves on the 30- and the 60-mg. levels of iron were not significant. An analysis of the linear regression coefficients, measuring the average biweekly changes in the hemoglobin levels of the calves, did not reveal a significant trend associated with copper levels.

In Trial 2, in which the calves were allowed to become anemic before receiving supplemental iron and copper, the average rate of decline of hemoglobin per

TABLE 1

*Average daily gains of calves fed various combinations of supplemental iron and copper in a 3 × 3 factorial, Trials 1 and 2*

| Copper levels | Gains | | | |
|---|---|---|---|---|
| | 0 mg. Fe | 30 mg. Fe | 60 mg. Fe | Over-all mean |
| *(mg.)* | | *(lb.)* | | |
| 0 | 1.77[a] | 2.24 | 1.87 | 1.95 |
| 3 | 1.56 | 1.93 | 1.95 | 1.82 |
| 6 | 1.56 | 2.02 | 2.12 | 1.90 |
| Over-all mean | 1.65 | 2.07 | 1.98 | —— |

[a] Each value is a mean for three calves.

2 wk. was 0.561 g. per 100 ml. The hemoglobin of the calves fed either 30 or 60 mg. of iron increased from an average of 5.4 to approximately 8.0 g. per 100 ml. blood in 16 to 20 wk. (Figure 2). The response was essentially the same for both groups. Hemoglobin levels in Trial 2 also were found not to be associated with copper level in the diet. The average linear regression coefficients indicating biweekly changes of calves receiving 0, 3, and 6 mg. copper per day (excluding those on the 0 level of Fe) were + 0.280, + 0.295, and + 0.282 g. hemoglobin per 100 ml. blood.

*Cell volume.* Hematocrit values paralleled, in general, hemoglobin values. During the depletion period in Trial 2, the hematocrit values dropped from an average of 40% to a low of about 20%, and when the iron supplements were added to the basal diet, the values rose proportionately with the increase in hemoglobin.

*Serum iron.* Serum-iron determinations were begun on the 8th wk. of the experimental period in Trial 1. Serum iron of the calves on the 0 level of iron varied from 20 to 40 γ per 100 ml. (Figure 1). The serum-iron values of the calves fed the 30- and the 60-mg. levels of iron were similar until the 22nd wk., fluctuating between 120 and 280 γ per 100 ml.; during the remainder of the ex-

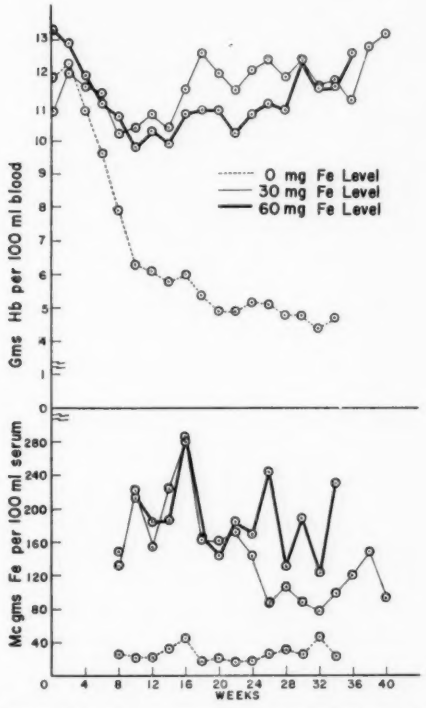


FIG. 1. Changes in hemoglobin and in serum-iron concentrations of blood from nine calves fed three levels of iron in Trial 1. (Each point is a mean of values from three calves.)

periment, the serum-iron values of the group receiving the 60-mg. level appeared to be higher than those of the group on the 30-mg. level of iron (Figure 1).

In Trial 2, the serum-iron values of the groups of calves fed the 30- and the 60-mg. levels of iron were similar, but were lower than those obtained from the same levels of iron in Trial 1 (Figure 2). Analysis of the biweekly changes in the serum iron of the calves on the 30- and the 60-mg. supplemental intakes of iron did not reveal significant differences associated with copper level in the diet, e.g., the average over-all biweekly changes associated with 0, 3, and 6 mg. of copper were −3.7, +0.6, and +1.2 γ per 100 ml., respectively.

*Serum copper.* Serum copper varied with dietary copper, on the average (Figure 3). Values as low as 10 mg. of copper were encountered among the animals on the 0 level of supplemental copper in Trial 2.

*Utilization of iron.* Since the absorbed iron is utilized almost completely for hemoglobin formation when animals are anemic *(10, 13, 15, 18)*, the total amount of iron utilized by the calves in this experiment can be considered to be approximately equal to the physiological requirement of iron necessary for the hemoglobin maintained and synthesized during an experimental period. The

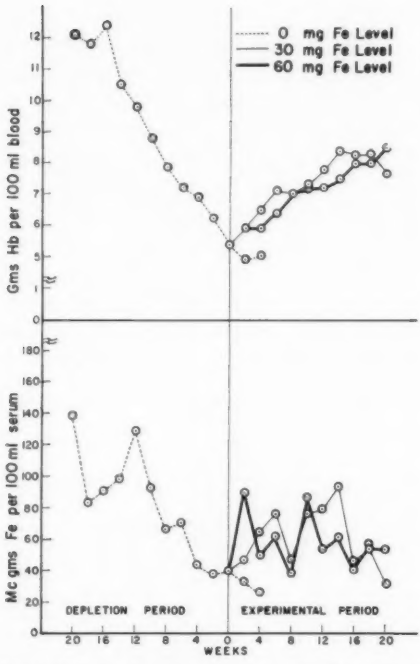


FIG. 2. Changes in hemoglobin and serum-iron concentrations of blood from 18 calves fed three levels of iron in Trial 2. (The depletion period varied from 9 to 20 wk. for individual calves. Each point in the experimental period represents a mean of values from a maximum of six calves.)

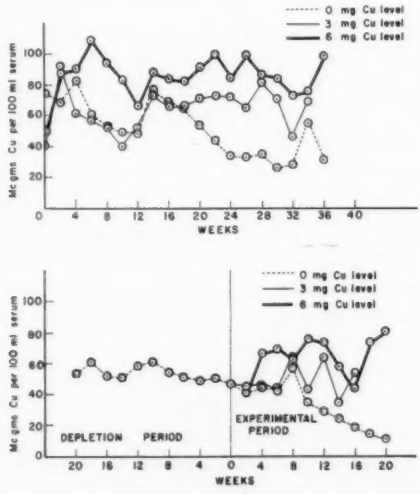


FIG. 3. Changes in serum-copper of calves fed three levels of copper in Trial 1 (above) and in Trial 2 (below).

total physiological requirement of iron may be expressed as a function of *(a)* iron needed to replace loss associated with the hemoglobin turnover and *(b)* amount of iron in hemoglobin synthesized *de novo*, or, more specifically, as follows:

$$P \text{ (mg.)} = \text{total hemoglobin maintained (g.)} \times \frac{d}{100} \times 0.09 \quad \text{(1-a)}$$

$$+ \text{ total hemoglobin synthesized (g.)} \times 0.335 \quad \text{(1-b)}$$

where

$P$ = total physiological iron requirement in mg.

$d$ = No. of days in experimental period

$\frac{1}{100}$ = turnover rate of hemoglobin per day [estimated from data reported by Drabkin *(10)*]

0.09 = mg. of iron needed to rebuild 1 g. of hemoglobin broken down internally *(10)*

0.335 = mg. of iron needed to synthesize *de novo* 1 g. hemoglobin, based on iron content of 1 g. hemoglobin *(10)*

Total hemoglobin was estimated by multiplying the blood volume [7] by the hemoglobin concentration. In calculating the physiological iron requirement for maintenance, the average value of the hemoglobin maintained during an experimental period was obtained by dividing the sum of the initial and final amounts of hemoglobin by 2. The hemoglobin synthesized during any experimental period was calculated by subtracting the initial hemoglobin from the final amount obtained at the end of the given experimental period. The per cent of dietary iron utilized *(U)* was determined as follows:

$$U = \frac{P}{D} \quad (2)$$

where

$P$ = value derived from Equation 1

$D$ = mg. dietary iron from supplement and basal diet

The mean per cent utilization of iron by the calves on the 30-mg. level was almost twice that of those on the 60-mg. level, 59% and 31%, respectively (Table 2). An analysis of variance of the utilization values indicated that copper did not have a significant effect ($P \geqslant .05$) on iron utilization (Table 2).

## DISCUSSION

Calves receiving either 30 or 60 mg. of iron per day throughout the experimental period (Trial 1) maintained a normal level of hemoglobin *(6, 23)*, indicating that the lower level of supplemental iron meets the requirement of calves fed a milk diet which contains approximately 1 p.p.m. of iron on a dry matter basis. For calves that had been depleted, the addition of either 30 or 60 mg. of

[7] Blood volume (ml.) = (Body weight)$^{0.93} \times 40.82$, derived from data reported by Brody *(5)* and Turner and Herman *(32)*.

TABLE 2

*Summary of average iron utilization by calves given various levels of supplemental copper and iron, Trials 1 and 2*

| Copper levels | Average utilization of iron | | |
|---|---|---|---|
| | 30 mg. Fe | 60 mg. Fe | Mean |
| *(mg.)* | | *(%)* | |
| 0 | 56.50 | 32.27 | 44.38 |
| 3 | 63.57 | 31.27 | 47.42 |
| 6 | 57.46 | 29.33 | 43.4 |
| Mean | 59.18 | 30.95 | |

iron to the diet caused a general increase in hemoglobin concentrations of the blood, indicating that the supplemental levels of iron were sufficient to meet the demands for growth as well as to contribute to accretion.

The calves did not attain normal hemoglobin values within the span of the experimental period, which was terminated when individual calves showed symptoms of the so-called milk syndrome. The similarity in hemoglobin response of the calves receiving the 30- and the 60-mg. levels of iron during the repletion period indicates that the total utilization of iron was the same for the respective levels. The calculated utilization values, based on considerations of the physiological demands for iron, gave confirmatory evidence that the total amount of iron absorbed was approximately the same for either level of iron intake.

These results are in agreement with the work of Hahn *et al. (14)*, who reported that dogs utilized as much as 50% of a small dose of iron (40 mg.) and only 5% of a large dose (400 mg.), but do not agree with studies by Balfour *et al. (1)*, by Copp and Greenberg *(8)*, and by Hahn *(12)*, who reported that in anemic dogs, rats, and humans the efficiency of iron utilization increased. The lack of agreement between the data of the latter workers, and the data from this investigation may be explained by the probability that the need for iron to meet the demand for growth alone (regardless of level of hemoglobin in blood) was great enough to maximize the absorption of dietary iron. For the mature animal, however, the degree of anemia is probably the greatest single factor determining the need for iron.

Either the 30- or the 60-mg. supplementation of iron was sufficient to maintain a normal level of serum iron *(23)* in Trial 1, but not in Trial 2. Since the animals in Trial 2 were depleted and, therefore, the demand for iron was for repletion as well as for growth, it is probable that the iron-deficient tissues of the depleted animals were constantly drawing iron from the plasma, resulting in low serum-iron values. These data, together with the hemoglobin and utilization data, suggest that the factor limiting the amount of hemoglobin synthesized was absorption of iron rather than its utilization after it had entered the blood circulation of the animal. These data suggest further the possibility that the absorption of iron, within physiological limits, may be dependent on a "carrier" system, and that the amount of iron absorbed is limited by the capacity of the carrier.

Based on the data presented herein, an estimate of the minimum nutritional

iron requirement of calves appears to be approximately 30 mg. per day. A consideration of the physiological needs of a calf indicates that the greater part of the iron is needed for growth. For example, the calculated physiological iron required to maintain a level of 10 g. of hemoglobin per 100 ml. blood in a 500-lb. calf (Equation 1-a) is 1.2 mg. per day. For a calf growing at the rate of 2 lb. per day, the physiological iron requirement for growth (Equation 1-b) is approximately 16 mg. iron per day. These calculations indicate that the average daily gain during growth, rather than size of the animal, is the major factor determining the amount of iron required.

Under the conditions of this investigation, the only effect noted in calves without copper supplementation was low serum-copper concentrations. The concentration of copper in the basal milk diet was 75 γ per liter of diet, or approximately 0.5 p.p.m. of the diet on the dry-matter basis. Since level of dietary copper had no significant effects on hemoglobin levels, presumably the copper in the basal diet and in the body stores was sufficient to sustain hemoglobin production at least for the periods of this investigation. The serum-copper data further indicate that 6 mg. of copper fed daily is sufficient to maintain or increase serum-copper concentrations to normal values *(23)*.

## SUMMARY

Twenty-seven male Holstein calves were used in a study to determine iron and copper requirements. The basal diet consisted of whole milk, supplemented with vitamins A and D, cobalt, and magnesium. The investigation was conducted in two trials, in which three levels of supplemental iron, 0, 30, and 60 mg. per day, and three levels of supplemental copper, 0, 3, and 6 mg. per day, were studied in a $3 \times 3$ factorial design involving a total of nine treatments. In the first trial, nine calves were started on the experimental treatments at 2 days of age. In the second trial, 18 calves, two replications, were fed the basal diet until the hemoglobin level declined to 5-6 g. per 100 ml. of blood, and then were started on the experimental treatments. The results were as follows:

1. Animals on the zero level of supplemental iron became anemic and gained less than those on the 30 mg. and the 60 mg. intakes of iron per day.
2. Calves fed either 30 mg. or 60 mg. of iron in Trial 1 maintained normal levels of hemoglobin and of red-cell volume. The hemoglobin level of calves receiving supplemental iron in Trial 2 increased from a mean value of 5.4 g. to 8.0 g. per 100 ml., during a period of from 16 to 20 wk. No difference was obtained in hemoglobin levels between the 30 mg. and the 60 mg. levels of iron intake.
3. Hemoglobin levels and calculated utilization values indicated that on the average approximately 60% of the 30 mg. level and 30% of the 60 mg. level of iron were utilized.
4. The data from this investigation indicate that the minimal nutritional iron requirement for maintenance of a normal hemoglobin level in growing calves is approximately 30 mg. per day.

5. Without supplemental copper in the diet, the serum-copper decreased, but an intake of 6 mg. of copper per day was sufficient to restore or to maintain a normal level of serum copper. Different dietary levels of copper did not influence hemoglobin concentration under the conditions of the experiment.

## REFERENCES


(1) Balfour, W. M., Hahn, P. F., Bale, W. F., Pommerenke, W. T., and Whipple, G. H. Radioactive Iron Absorption in Clinical Conditions: Normal, Pregnancy, Anemia, and Hemochromatosis. *J. Expl. Med.*, **76**: 15. 1942.

(2) Becker, R. B., Arnold, P. T. D., Kirk, W. G., Davis, G. K., and Kidder, R. W. Minerals for Dairy and Beef Animals. *Univ. Fla. Agr. Expt. Sta., Bull.* **513**: 5. 1953.

(3) Bennetts, H. W., Beck, A. B., Harley, R., and Evans, S. T. "Falling Disease" of Cattle in the South-West of Western Australia. II. Studies of Copper Deficiency of Cattle. *Australian Vet. J.*, **17**: 85. 1941. (From *Chem. Abstr.*, **35**: 8047. 1941. Original not seen.)

(4) Bergeim, O., and Kirch, E. R. Reduction of Iron in the Human Stomach. *J. Biol. Chem.*, **177**: 591. 1949.

(5) Brody, S. *Bioenergetics and Growth.* Reinhold Publ. Corp., New York. 1945.

(6) Brooks, J. H., and Hughes, J. S. The Hemoglobin Content of the Blood of Dairy Cattle. *J. Nutrition*, **5**: 35. 1932.

(7) Cochran, W. G., and Cox, G. M. *Experimental Designs.* John Wiley and Sons, Inc., New York. 1952.

(8) Copp, D. H., and Greenberg, D. M. A Tracer Study of Iron Metabolism with Radioactive Iron. *J. Biol. Chem.*, **164**: 377. 1946.

(9) Davis, G. K., Kidder, R. W., and Comar, C. L. Copper Deficiency in Cattle. *J. Animal Sci.*, **5**: 393. 1946.

(10) Drabkin, D. L. Metabolism of the Hemin Chromoproteins. *Physiol. Revs.*, **31**: 345. 1951.

(11) Gubler, C. J., Taylor, D. S., Eichwald, E. J., Cartwright, G. E., and Wintrobe, M. M. Copper Metabolism. XII. Influence of Manganese on Metabolism of Copper. *Proc. Soc. Exptl. Biol. Med.*, **86**: 223. 1954.

(12) Hahn, P. F. Metabolism of Iron. *Fed. Proc.*, **7**: 493. 1948.

(13) Hahn, P. F., Bale, W. F., Lawrence, E. O., and Whipple, G. H. Radioactive Iron and Its Metabolism in Anemia. Its Absorption, Transportation, and Utilization. *J. Exptl. Med.*, **69**: 739. 1939.

(14) Hahn, P. F., Bale, W. F., Ross, J. F., Balfour, W. M., and Whipple, G. H. Radioactive Iron Absorption by Gastro-intestinal Tract. *J. Exptl. Med.*, **78**: 169. 1943.

(15) Hahn, P. F., Ross, J. F., Bale, W. F., and Whipple, G. H. The Utilization of Iron and the Rapidity of Hemoglobin Formation in Anemia Due to Blood Loss. *J. Exptl. Med.*, **71**: 731. 1940.

(16) Hawkins, G. E. Manganese, Calcium and Phosphorus Interrelationships in the Nutrition of Dairy Calves. Thesis, N. C. State College Library. 1952.

(17) Henderson, L. M., McIntire, J. M., Waisman, H. A., and Elvehjem, C. A. Pantothenic Acid in the Nutrition of the Rat. *J. Nutrition*, **23**: 47. 1942.

(18) Joseph, H. W. Studies on Iron Metabolism and the Influence of Copper. *J. Biol. Chem.*, **96**: 559. 1932.

(19) Kirch, E. R., Bergeim, O., Kleinberg, J., and James, S. Reduction of Iron by Foods in Artificial Gastric Digestion. *J. Biol. Chem.*, **171**: 687. 1947.

(20) Kitzes, G., Elvehjem, C. A., and Schuette, H. A. The Determination of Blood Plasma Iron. *J. Biol. Chem.*, **155**: 653. 1945.

(21) Kletzien, S. W. Iron Metabolism. 1. The Role of Calcium in Iron Assimilation. *J. Nutrition*, **19**: 187. 1940.

(22) Knoop, C. E., Krauss, W. E., and Washburn, R. G. The Development of Nutritional Anemia in Dairy Calves. *J. Dairy Sci.*, **18**: 337. 1935.

(23) MATRONE, G., PETERSON, W. J., BAXLEY, H. M., AND GRINNELLS, C. D. Copper and Iron in the Blood Serum of Dairy Calves. *J. Dairy Sci.*, **30**: 121. 1947.

(24) MITCHELL, H. H. Mineral Requirements of Farm Animals. *J. Animal Sci.*, **6**: 365. 1947.

(25) MITCHELL, H. S., AND MILLER, LILA. Studies in Nutritional Anemia. *J. Biol. Chem.*, **92**: 421. 1931.

(26) PARKS, R. Q., HOOD, S. L., HURWITZ, C., AND ELLIS, G. H. Quantitative Chemical Microdetermination of Twelve Elements in Plant Tissue. *Ind. Eng. Chem., Anal. Ed.*, **15**: 527. 1943.

(27) RAGSDALE, A. C., AND BRODY, S. Growth and Development. Growth and Development of Dairy Cattle. *Missouri Agr. Expt. Sta., Research Bull.*, **96**: 8. 1926.

(28) SHENK, J. H., HALL, J. L., AND KING, H. H. Spectrophotometric Characteristics of Hemoglobin. I. Beef Blood and Muscle Hemoglobins. *J. Biol. Chem.*, **105**: 741. 1934.

(29) SMITH, S. E., AND ELLIS, G. H. Copper Deficiency in Rabbits. Achromotrichia, Alopecia and Dermatitis. *Arch. Biochem.*, **15**: 81. 1947.

(30) THOMAS, J. W., OKAMOTO, M., JACOBSON, W. C., AND MOORE, L. A. A Study of Hemoglobin Levels in the Blood of Young Dairy Calves and the Alleviation of Anemia by Iron. *J. Dairy Sci.*, **37**: 805. 1954.

(31) TOMPSETT, S. L. Factors Influencing the Absorption of Iron and Copper from the Alimentary Tract. *Biochem. J.*, **34**: 961. 1940.

(32) TURNER, C. W., AND HERMAN, H. A. Determination of the Blood and Plasma Volume of Dairy Cattle. *Missouri Agr. Expt. Sta., Research Bull.*, **59**: 1. 1931.

# EFFECT OF DIPHENYL-*p*-PHENYLENEDIAMINE ON THE UTILIZATION OF CAROTENE AND VITAMIN A BY THE CALF [1,2]

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**The toxicity of diphenyl-*p*-phenylenediamine (DPPD) and its effect on the absorption of vitamin A and carotene from the gut of the dairy calf was investigated. Growth was depressed when DPPD was first fed, but thereafter it had little effect on the health of calves as indicated by weight, blood cell counts, hemoglobin levels, and general appearance. High levels of DPPD (3 to 10 g/100 lb. of body weight) impaired vitamin A absorption, whereas lower levels (250 p.p.m.) improved absorption of this vitamin and of carotene.** *Editor.*

Diphenyl-*p*-phenylenediamine (DPPD) has been found to reduce the loss of carotene in dehydrated alfalfa meal and, for a period of time, was used for this purpose in the feed industry. In chickens, it has been found to prevent encephalomalacia *(5)* and to improve the utilization of vitamin A *(11)* and of carotene (*10*), although in other research (*3*) DPPD was found ineffective with regard to the latter. DPPD failed to improve carotene absorption in an experiment with rats *(7)*, although it was the only antioxidant studied which did not impair carotene absorption at the relatively high dietary concentrations employed. Carotene utilization was improved by inclusion of DPPD in the diet of cows on low carotene rations *(17)* and in the diet of calves fed alfalfa *(14)*. The present study was carried out to determine the toxicity of DPPD and its effect upon the absorption of vitamin A and carotene from the gut of the dairy calf.

## EXPERIMENTAL PROCEDURE

The effect of DPPD upon utilization of vitamin A and $\beta$-carotene was investigated in two ways. First, blood plasma levels of vitamin A and carotenoids were used as an indication of the effect of DPPD upon the absorption of these vitamin substances. Secondly, a post-supplementation depletion period was employed to estimate carotenoid and vitamin A storage. Pertinent data relative to the experimental animals and procedures are shown (Table 1). Calves receiving a high level of DPPD (Trials I and V) were used to evaluate the toxicity of this compound.

The calves of Trials I, II, and III were allotted either to the DPPD or to the control subgroup at the beginning of the trials, and received the same treatment throughout the whole experimental period. In Trial IV, however, the calves were allotted anew before each dosing.

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TABLE 1

*Data relative to experimental animals and procedures*

| Trial | | No. and breed of calves[a] | DPPD administration | | Absorption phase | | | Depletion phase | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Rate[b] | Mode[c] | Vitamin suppl. | Av. age at start | Exptl. period | Av. age at start | Exptl. period |
| | | | | | | *(days)* | *(days)* | *(days)* | *(days)* |
| I | C | 4H | —— | —— | Vitamin A | 96 | 29 | 125 | 196 |
| | D | 4H | 3-10g[d] | conc. mix. | Vitamin A | 101 | 29 | 130 | 196 |
| II | C | 1H, 1G | —— | —— | Vitamin A | 4 | 46 | 50 | 133 |
| | D | 1H, 1G | 250 p.p.m. | capsule | Vitamin A | 4 | 46 | 50 | 168 |
| III | C | 1H, 1J | —— | —— | Carotene | 4 | 42 | 46 | 66 |
| | D | 1H, 1J | 250 p.p.m. | capsule | Carotene | 4 | 42 | 46 | 70 |
| IV | C | 3H | —— | —— | Carotene | 71 | | | |
| | D | 3H | 250 p.p.m. | capsule[e] | Carotene | 88 | | | |
| V[f] | D | 2H, 1J, 1G | 3g. | capsule and conc. mix. | (toxicity study only) | | | | |

[a] H = Holstein, G = Guernsey, J = Jersey.
[b] g. = daily rate/100 lb. body weight; p.p.m. = level in feed.
[c] Capsules were fed while calves were drinking from nipples.
[d] 3 to 10 g. in absorption trial; 3 g. in depletion trial.
[e] Feeding started 4 days before, and continued through, evening following carotene administration.
[f] Average age at beginning, 109 days; length of experimental period, 76 days.

The feeding and management of the experimental calves in the absorption trials followed the herd routine. The young calves (Trials II and III) received Holstein whole milk and, starting at three weeks of age, calf starter and alfalfa hay were fed to all ruminating calves except to those on the depletion experiment. They were weaned at from 6 to 7 wk. of age. The depletion ration consisted of 1-year-old oat straw and a concentrate mixture composed of 50% oats, 37% wheat bran, 10% linseed oil meal, 1% each of iodized salt, steamed bonemeal, and calcium carbonate, and a trace of irradiated yeast.

The calves were weighed weekly, and body temperature and consistency of feces were checked daily. The calves were observed closely for symptoms of toxicity or other abnormalities. Erythrocyte and leucocyte counts of blood from calves fed high levels of DPPD, and their controls, were made at weekly intervals throughout the first 10 wk. of the experiment, and less frequently thereafter. Hemoglobin determinations were made occasionally.

The effect of DPPD upon the absorption of vitamin A and carotene was estimated from the blood plasma levels of vitamin A and carotenoids at 0, 3, 6, 9, 12, and 15 hr. subsequent to the administration of a massive dose (100,000 IU/100 lb. body weight) of vitamin A[3] or β-carotene.[4] A similar procedure was

[3] Vitamin A acetate in gelatin (250,000 USP units/g) was obtained from Chas. Pfizer and Co. Inc., through the courtesy of W. M. Reynolds.

[4] 24% β-carotene suspension in vegetable oil (400,000 USP units/g) was obtained from Hoffmann-La Roche, Inc., through the courtesy of G. K. Parman.

employed successfully in previous vitamin A and carotene absorption studies *(9)*. Absorption curves were determined three times for each calf in Trial I and four times in Trials II and III. In Trial IV six calves were used to measure the effect of DPPD on the absorption of carotene, as estimated by changes in blood plasma carotenoid levels. Subsequent to the absorption study of Trials I, II, and III, the calves were employed in a depletion trial, to test the effect of DPPD on vitamin A storage. The technique was similar to one described earlier *(8)*. When the plasma level of vitamin A had decreased to an average of about 8 $\gamma$/100 ml. for two consecutive weeks, the calves from Trials II and III were repleted with 100,000 IU/100 lb. body weight of vitamin A (Trial II) or carotene (Trial III). Absorption curves were determined during the 15 hr. subsequent to repletion.

The analysis for vitamin A and carotenoids was performed according to the method of Allen *et al. (2)*. The DPPD in the plasma was estimated by a modification of the method of Teichman *et al. (17)*. The Skelly A extract of the plasma was evaporated and the residue was dissolved in acetone. Four drops of concentrated nitric acid were added and optical density at 490 m$\mu$ was measured with a Beckman Spectrophotometer, Model DU. The quantity of DPPD present was estimated from a standard curve. In addition, the more specific, but less sensitive, method of Smith *et al. (16)* was employed to ascertain the presence of DPPD itself.

## RESULTS AND DISCUSSION

There was some indication of a temporary growth retardation for periods up to about 3 wk., after feeding of DPPD at high levels (3 to 10 g/100 lb. body weight) was initiated but, subsequently, growth was satisfactory. The blood counts showed considerable variation, but no consistent difference was detectable between experimental and control calves. After 6 mo. on high levels of DPPD, the experimental calves had 8.6 million erythrocytes and 6,000 leucocytes per cmm. of blood, whereas the control calves had 8.6 million and 6,900, respectively. The hemoglobin content of the experimental calves was 12.8 g/100 ml. blood.

One calf died and three animals were sacrificed, because of various abnormalities, during the course of this experiment. Although three of these animals had been fed a high level of DPPD, necropsy did not reveal any symptoms which would be indicative of DPPD intoxication.

There was little indication of harm from DPPD, even at the comparatively high dietary levels employed, and there was no evidence of anemia which Seifter *(15)* considers to be a major symptom of intoxication by aromatic amines. The results agree with those of Matterson *et al. (12)*, who could not detect deleterious effects when chickens were fed 1% DPPD in the ration. It seems probable that the low solubility of DPPD is mostly responsible for the comparatively low toxicity.

Feeding of DPPD at high levels to ruminating calves (Trial I) resulted in a decrease in rate of absorption of vitamin A (Figure 1). When analysis of variance was employed to evaluate the maximum plasma levels, the difference between treatments was found to approach significance at $P = 0.05$. Maximum blood plasma levels of vitamin A occurred at 3 hr. for each group. No appreci-

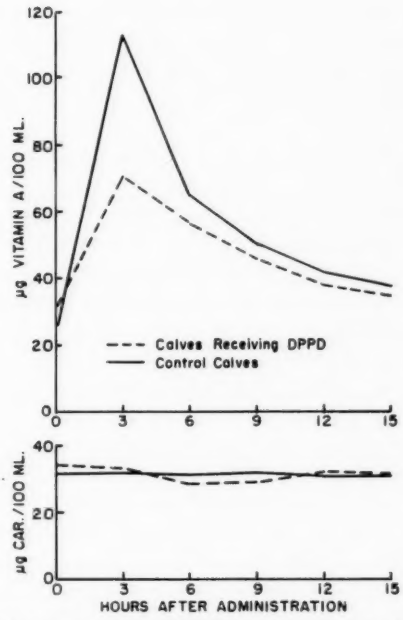


FIG. 1. Influence of high levels of dietary DPPD upon mean blood plasma levels of vitamin A and carotenoids, following the oral administration of a single massive dose of vitamin A (Trial I); four calves per group, three absorption trials per calf.

able change in blood plasma carotenoid values was observed. Feeding of DPPD at the rate of 250 p.p.m. in the feed of young calves (nondepleted) had little effect upon rate of absorption of vitamin A (Fig. 2). However, limited data indicate that at the end of the depletion period, DPPD improved vitamin A absorption in the same calves, following administration of massive doses of vita-

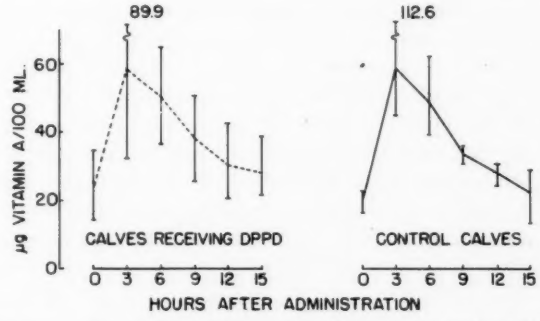


FIG. 2. Effect of a low level of DPPD upon mean blood plasma levels of vitamin A, following administration of massive doses of this vitamin (Trial II). Vertical bars indicate range of values; two calves per group, four absorption trials per calf.

min A. While interference with carotene absorption by high doses of dietary antioxidants is rather frequently reported, only one communication *(13)* indicates that antioxidants interfere with vitamin A absorption. The cause of the apparent decrease in absorption of vitamin A in the present experiment is not clear.

The vitamin absorption measurements of Trial II were not performed on the same day for calves within pairs, which might account in part for the relatively large variations observed. The intrinsic variation of the procedure for analysis of plasma vitamin A and carotenoids seems to be rather large *(6)* and, in particular, samples taken on different days from the same calf may vary significantly *(1)*. When the absorption curves were performed simultaneously within the replicate, DPPD in low dietary concentrations seemed to enhance the vitamin A absorption, which is in accord with results in poultry *(11)*, although in the present experiment the use of vitamin A in a presumably easily absorbable preparation would make any protective effect less conspicuous.

Contrary to earlier observations *(9)*, no increase in blood plasma carotenoid levels occurred up to 24 hr. after administration of β-carotene in gelatin capsules (Trial III). This apparent lack of absorption was first attributed to the high concentration of the carotene preparation (semi-solid suspension in vegetable oil, 400,000 USP units/g). However, neither dilution with corn oil nor dilution with Tween 80[5] resulted in improvement of carotene absorption. Increased uptake occurred, however, when the β-carotene was dissolved in melted lard and the lard was homogenized into skimmilk.

Since a substantial part of Trial III as originally designed had been completed before a satisfactory mode of carotene administration was developed, additional animals (Trial IV) were assigned to the carotene absorption study. Figure 3 shows the effect of DPPD on blood plasma carotenoid and vitamin A levels, subsequent to administration of massive doses of β-carotene. The values in Figure 3 represent the averages for the initial absorption curves of three pairs of calves. Subsequently, an additional ten absorption curves (five with DPPD and five without) were obtained, employing these same animals. The results were not appreciably different from those shown; the values were not employed in deriving the means, because of variations in age and in number of absorption curves per animal. Analysis of covariance did not reveal any dependence of the rate of increase of plasma carotenoids upon the initial level. The rather definite effect of DPPD on carotene absorption as compared to vitamin A absorption is not unexpected. Apparently, carotene is more sensitive to influences in the intestinal tract and, therefore, may respond more than vitamin A to any improvement. In addition, the error associated with carotene determination seems to be smaller *(6)*.

The calves of Trials II and III were depleted subsequent to the absorption trials. The depletion times varied considerably, but were somewhat longer for animals fed DPPD. The absorption curves resulting from repletion of the de-

[5] Polyoxyethylene sorbitan monooleate supplied by the Atlas Powder Co., Wilmington, Delaware.

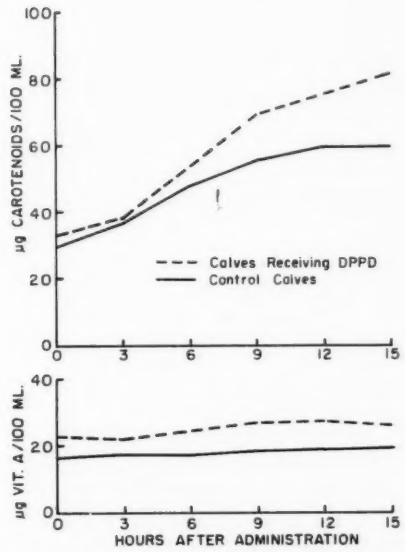


FIG. 3. Influence of low levels of dietary DPPD upon mean blood plasma levels of vitamin A and carotenoids, following the administration of a single massive dose of β-carotene (Trial IV); three calves per group, one absorption trial per calf.

ficient calves indicated improved absorption of carotene and vitamin A in DPPD-fed calves.

The eight calves of Trial I were depleted; four were on a high level of DPPD. The mean blood plasma levels of vitamin A and carotenoids (Figure 4) were consistently higher throughout the depletion period for animals fed DPPD. There was a gradual downward trend in plasma vitamin A values; however, at one stage a rather sharp increase in carotenoid levels occurred, accompanied by a temporary rise in vitamin A values. Since the forage provided was 1-year-old oat straw, the increase in plasma carotenoids seemed rather surprising. Analysis of the components of the ration indicated an intake of carotene of approximately 16,000 IU/day when 10 lb. of straw were consumed daily. Although the efficiency of absorption of carotene from straw probably is lower than from more conventional calf feeds, it must have been the primary source of dietary carotene. Furthermore, even though vitamin A stores at the beginning of depletion may have been relatively high, it is likely that the carotene derived from the straw was largely responsible for the prolonged depletion period. A considerable dietary uptake is further substantiated by the decline of the plasma carotenoid levels after reduction of the straw allowance to one-half the previous rate after 20 wk.

It is reported *(4)* that DPPD interferes with the carotene determination in alfalfa meal. When a feed-grade DPPD was used in in vitro tests, no interference with the carotenoid analysis by the color test employed in this study could be

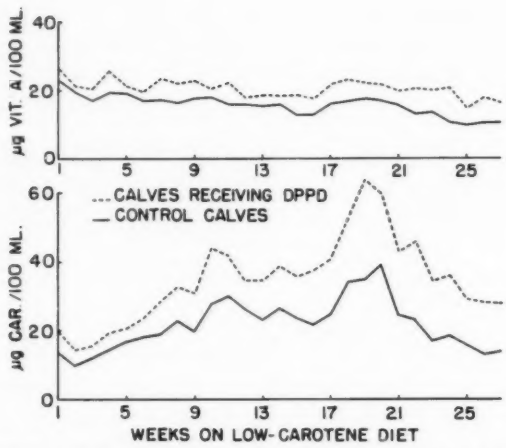


FIG. 4. Influence of high levels of dietary DPPD upon mean blood plasma levels of vitamin A and carotenoids during a period on a low carotene diet (Trial I).

detected, but the presence of DPPD increased the readings obtained for vitamin A. This interference could account, at least in part, for the higher plasma vitamin A levels of calves fed DPPD. However, in vivo observations failed to demonstrate any effect of DPPD on vitamin A values. The effect, if any, on carotene determination is not clear. The relationship between plasma levels of vitamin A, carotenoids, and apparent DPPD, during DPPD feeding and after DPPD removal from the diet, is presented (Table 2). The apparent DPPD levels were estimated colorimetrically by reaction of nitric acid with an acetone solution of the materials originally extracted from plasma with Skellysolve A, a procedure which is a modification of the method of Teichman *et al. (17)* for determining DPPD in milk. It should be noted that the analysis for DPPD was very approximate and may include several related (or unrelated) compounds which tend to complicate evaluation.

TABLE 2

*Effect of dietary DPPD on the blood plasma levels of vitamin A, carotenoids, and apparent DPPD*

| Date | Vitamin A | | | Carotenoids | | | Apparent DPPD |
|---|---|---|---|---|---|---|---|
| | Experimental[a] | Control[b] | Difference | Experimental[a] | Control[b] | Difference | |
| | | | | *(γ/100 ml.)* | | | |
| 1-14-56 | 14.9 | 9.8 | 5.1 | 29.1 | 15.8 | 13.3 | 150 |
| 1-28-56 | 16.4 | 10.4 | 6.0 | 27.6 | 14.8 | 12.8 | 142 |
| 2-4-56[c] | 14.4 | 8.5 | 5.9 | 23.9 | 11.4 | 12.5 | 74 |
| 2-9-56 | 14.3 | 8.0 | 6.3 | 18.1 | 10.4 | 7.8 | 30 |
| 2-11-56 | 15.9 | 11.7 | 4.2 | 22.9 | 15.1 | 7.8 | 24 |

[a] Each value is an average of four animals.

[b] Each value through 2-4-56 is an average of four animals; each subsequent value is an average of two.

[c] DPPD discontinued.

The uptake, as well as the excretion, of DPPD seems to occur fairly rapidly, as indicated by the apparent DPPD levels during and following discontinuation of DPPD feeding (Trial I). This is even more pronounced in the data (Figure 5), which summarize results of a short trial involving two calves. One calf received 10 g. DPPD/100 lb. body weight per day for 3 wk. and the other received none. Since the calf fed no DPPD exhibited an appreciable "apparent DPPD" level, the presence of other compounds, which absorb at 490 mμ and thus complicate evaluation, is indicated. It is significant that the more specific but less sensitive method of Smith *et al. (16)* indicated the presence of DPPD in only one (highest value, Figure 5) of the samples represented (Table 2 and Figure 5). Further work would be required to clarify some of the above relationships.

## SUMMARY

High dietary levels of DPPD (3 to 10 g/100 lb. body weight) were administered to dairy calves in an attempt to assess the toxicity of this antioxidant. There was an indication of a depression of growth when DPPD was first introduced into the diet; thereafter, the DPPD apparently had little effect upon the health of the calves. Criteria used were weight gains, blood cell counts, hemoglobin levels, and general health observations.

The absorption curve technique was employed to study the effect of DPPD upon the absorption of vitamin A and carotene. At the high dietary levels used in the study of toxicity, DPPD impaired the absorption of vitamin A. When DPPD was fed at lower concentrations (250 p.p.m. in the diet), there was an indication that DPPD improved the absorption of vitamin A slightly. At the lower dietary concentration, DPPD improved the absorption of carotene.

In the course of a long-term depletion experiment, it became evident that DPPD or some derivative thereof was absorbed from a diet containing a high level of DPPD (3 g/100 lb. body weight). The absorbed DPPD or DPPD product probably interfered with the analytical technique and thus caused an ap-

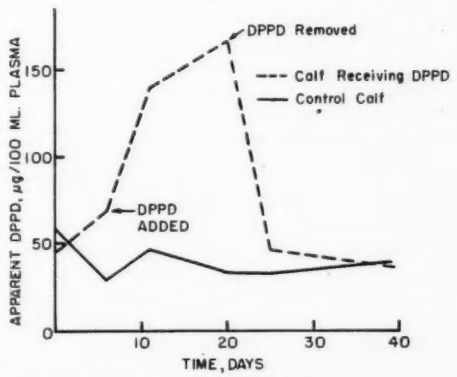


FIG. 5. Effect of ingestion of a high level of DPPD upon the apparent DPPD levels in blood plasma.

parent increase in the plasma levels of vitamin A and carotenoids. When DPPD feeding was discontinued, a sudden drop in the plasma level of apparent DPPD occurred, indicating rapid metabolism of this compound.

## REFERENCES


(1) Allen, R. S., Homeyer, P. G., and Jacobson, N. L. Use of Activated Glycerol Dichlorohydrin for Estimating Vitamin A in Dairy Calf Blood Plasma. *Iowa State Coll. J. Sci.*, **29**: 721. 1955.

(2) Allen, R. S., Wise, G. H., and Jacobson, N. L. Occurrence in Blood Plasma from Certain Dairy Calves of Factors that Interfere with the Color Reactions of Activated Glycerol Dichlorohydrin with Vitamin A and Carotenoids. *J. Dairy Sci.*, **32**: 688. 1949.

(3) Almquist, H. J., and Maurer, S. The Effect of Antibiotic, Antioxidant, and Fat on Conversion of Carotene to Vitamin A in the Chicken. *Arch. Biochem. Biophys.*, **55**: 297. 1955.

(4) Beauchene, R. E., Mitchell, H. L., Parrish, D. B., and Silker, R. E. Carotene. Effect of Antioxidants on Determination in Dehydrated Alfalfa Meal. *J. Agr. Food Chem.*, **1**: 461. 1953.

(5) Bunell, R. H., Matterson, L. D., Singsen, E. P., Potter, L. M., Kozeff, A., and Jungher, E. L. Studies on Encephalomalacia in the Chick. III. The Influence of Feeding or Injecting Various Tocopherols and Other Antioxidants on the Incidence of Encephalomalacin. *Poultry Sci.*, **34**: 1068. 1955.

(6) Clayton, M. M., Babcock, M. J., Foster, W. D., Stregevsky, S., Tucker, R. E., Wertz, A. W., and Williams, H. H. A Referee Blood Experiment Involving the Use of Microchemical Methods. *J. Nutrition*, **52**: 383. 1954.

(7) High, E. G. Further Antioxidant Studies Concerned with the Metabolism of Carotene and Vitamin A. *Arch. Biochem. Biophys.*, **60**: 456. 1956.

(8) Jacobson, N. L., Allen, R. S., Blake, J. T., and Homeyer, P. G. The Effect of Method of Administration on the Absorption and Storage of Vitamin A by Dairy Calves. *J. Nutrition*, **54**: 143. 1954.

(9) Jacobson, N. L., Wise, G. H., Allen, R. S., and Kempthorne, O. Rate of Absorption of Carotene and of Vitamin A from the Alimentary Tract of Dairy Calves. *J. Dairy Sci.*, **33**: 645. 1950.

(10) Matterson, L. D. Antioxidants. *Proc. Nutrition Council*, Am. Feed Manufacturers Assoc., Chicago, Ill. November, 1955.

(11) Matterson, L. D., Bunell, R. H., Stinson, L. D., Singsen, E. P., and Potter, L. M. The Effect of Incorporating DPPD into a Ration Containing Various Levels of Vitamin A from Fish Oils. *Poultry Sci.*, **34**: 1080. 1955.

(12) Matterson, L. D., Potter, L. M., Kozeff, Anna, and Junghere, E. L. The Relative Nontoxicity of DPPD to Chicks when Fed Continuously for 12 Weeks at the 1% Level. *Poultry Sci.*, **34**: 239. 1955.

(13) Miles, M. C., Erickson, E. M., and Mattil, H. A. Tocopherol vs. Tocopherol Acetate as a "Sparer" of Vitamin A. *Proc. Soc. Exptl. Biol. Med.*, **70**: 162. 1949.

(14) Rousseau, J. E., Jr., Eaton, H. D., Teichman, R., Helmboldt, C. F., Junghere, E. L., Bacon, E. L., Dolge, K. L., Beall, G., and Moore, L. A. Effect of Some Antioxidants on the Utilization of Carotene by Holstein Calves. *J. Dairy Sci.*, **39**: 1671. 1956.

(15) Seifter, J. General Pharmacology and Toxicology of Quinones and Hydroquinones. *Biological Antioxidants, Trans. Third Conf.* p. 50. Josiah Macy, Jr. Foundation. New York. 1948.

(16) Smith, H., Beauchene, R. E., Parrish, D. B., and Mitchell, H. L. Detection of DPPD in Dehydrated Alfalfa and in Mixed Feeds. *J. Agr. Food Chem.*, **3**: 788. 1955.

(17) Teichman, R., Rousseau, J. E., Jr., Morgan, M. E., Eaton, H. D., McLeod, Patricia, Dicks, Martha W., and Johnson, R. E. Effect of Feeding Various Levels of N, N'-diphenyl-para-phenylenediamine to Lactating Dairy Cows and Its Detection in the Milk. *J. Dairy Sci.*, **39**: 1064. 1956.

# OBSERVATIONS ON THE DEGREE OF EXPRESSION OF ESTRUS IN CATTLE

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**The repeatability and heritability of heat symptoms and the relationship of heat to conception were investigated. The heritability of the expression of heat was 0.21. Heifers that conceived at first service had a rating for expression of heat of 0.22 unit higher, whereas, those that conceived at the second service had a rating of 0.17 unit higher, than those that failed to conceive. Both values were significant. The degree of expression of heat is useless in selecting for an increased conception rate.** *Editor.*

The detection of heat is difficult when its external symptoms are vague. As a consequence, heat periods may pass unnoticed, or animals may be bred at an unsuitable stage. Thus, conception may be delayed unnecessarily. In spite of its obvious practical importance, the degree of the expression of heat symptoms, and its relation to the chances of conception, have so far been given little attention in the studies of fertility in cattle.

Weber *(11)* made extensive observations on the different heat symptoms on 24 cows. He found that the strength of the heat symptoms was, to a large extent, determined by the individuality of the cows; consequently, he divided the cows into a group of 14 cows showing weak, nine showing medium, and one showing strong heat symptoms. He also found that the strength of the symptoms varied somewhat for the same cow from one heat period to another. In the 1954 Swedish artificial insemination work *(8)*, 229,392 cows were classified according to the strength of the heat symptoms. Of the total, 207,376 were classified as showing distinct, 2,955 as indistinct, and 1,389 as very indistinct heat symptoms. The percentages of diagnosed pregnancies in the three groups were approximately 57, 45, and 26, respectively. In making the classifications, the inseminator relied largely on the herdsman's judgment for the degree of expression of heat.

The objectives of the present study were to determine the repeatability and heritability of the degree of expression of heat symptoms, and to determine the relationship of the degree of expression of heat to conception.

## SOURCE AND DESCRIPTION OF DATA

The data were collected from the heifers at six Danish progeny stations during the testing years 1954-55 and 1955-56. Fifteen to 20 daughters by each of 29 different sires were assembled at the six stations the first of September, and

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were due to calve between the first of October and the 15th of November, at an age of 27 to 33 mo. During the testing year, the expression of heat was scored on 341 Red Danish heifers, 138 Black and White, and 75 Jerseys sired by 18 Red Danish bulls, seven Black and White, and four Jerseys. All the animals at a station were scored by one man, who restricted his scoring to animals at that one station.

The degree of expression of heat was evaluated according to the following scale, which ranges from 1 to 4:

1. The heat symptoms were so vague that the observer was not completely assured that the heifer was in heat.
2. The heat symptoms were weaker than usual, but the observer was not in doubt as to whether or not the heifer was in heat.
3. The heat symptoms were of normal strength.
4. The heat symptoms were more evident than normally.

To help describe the data, the observations on the daughters of a sire, "Rode," are shown (Table 1). The 18 daughters of Rode were tested at the Sundsgaard progeny-testing station in the year 1955-56. In explaining the figures and symbols, consider the daughter No. 9. On her first heat, No. 9 received a rating of 3, but was not inseminated. On her second heat, she received a rating of 3, was inseminated, but did not conceive. On her third heat, she received a rating of 3, was inseminated, and conceived.

The average number of observations per heifer was 3.04; whereas, the average number of observations per bull was 58.1, with a range of from 34 to 104. The average score per heifer was 2.46, and the average score per sire ranged from 1.82 for the 19 daughters of "Junker," to 2.93 for the 17 daughters of "Abild."

## ANALYSIS OF DATA ON EXPRESSION OF HEAT SYMPTOMS

The analysis of variance of the data is shown (Table 2). The difference between daughters of the same bull, between progeny groups at the same stations in the same year, and between station-years were all significant at the 1% level of significance. At the two stations Sundsgaard and Danstrupbundgaard, which rated the expressions of heat in both 1954-55 and 1955-56, there were large dif-

TABLE 1

*Example of the data assembled from the barn records*

| | | Daughters of the sire "Rode" | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Daughter No. ..... | | 1 | 2 | 3 | 4 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| Scores at the various heat periods | 1st Heat | 1+ | [a]4[b] | 3 | 3− | 2 | 3 | 4− | [c]3 | 2 | 2− | 1− | 2+ | 3+ | 2− | 3 | 3− | 3 | 3− |
| | 2nd Heat | | 4 | 1+ | 3− | 2+ | 2+ | 3+ | 3− | 2+ | 3− | 1+ | | | 3− | 2− | 1− | 3 | 3+ |
| | 3rd Heat | | 3+ | | 3+ | | | | 3+ | | 2− | | | | 3− | 1− | 3+ | 3+ | |
| | 4th Heat | | | | | | | | | | 2− | | | | 2− | 2+ | | | |
| | 5th Heat | | | | | | | | | | 2+ | | | | 2− | | | | |

[a] + Inseminated and conceived.
[b] The absence of + or − indicates that the cow was not serviced at that heat.
[c] − Inseminated but did not conceive.

ferences between the means of the groups tested at the same stations in the different years; consequently, it was necessary to remove the year-to-year differences at the same station by analyzing the data on a station-year basis. A station-year group is the group of heifers tested at the same station in the same year, and the differences between station-vear groups consist of both station differences and year-to-year differences at the same station. The station differences consist mainly of differences between progeny groups and differences caused by different men scoring the animals. The differences between years at the same station consist mainly of differences between progeny groups and differences caused by the way in which the same man interpreted the expression of heat in one year as compared to the other year. The differences between progeny groups at the same station should be free of station and man differences. It is conceivable that the scorer could have a tendency to discriminate against the daughters of one sire and be lenient toward those of another sire, but a large part of these data were collected early in the testing period, presumably before the scorer would have had time to develop a distinct like or dislike for any one group.

TABLE 2

*Analysis of variance of the ratings for the degree of expression of heat*

| Source | Degrees of freedom | Mean square | Components of variance |
|---|---|---|---|
| Total | 1,685 | | |
| Between station-years | 7 | 11.16++[a] | A = 0.041 |
| Between progeny groups within station-years | 21 | 2.48++ | B = 0.028 |
| Between daughters of the same bull | 525 | 0.77++ | C = 0.130 |
| Error | 1,132 | 0.38 | E = 0.380 |

[a]++ Significant at the 0.01 level of probability.

By equating the mean squares (Table 2) to their respective expected mean squares, as explained by Henderson's *(6)* Method 1, the Components of Variance A, B, C, and E are derived. The Component E is a measure of the variation between the scores of the same cow during the same breeding season and is environmental variance. The Component C is a measure of the variation caused by genetic and permanent environmental differences between the scores of the daughters of a sire in the same breeding season, and B is a measure of the variation caused by hereditary differences among the sires of the heifers. The Component B consists of one-fourth of the additive genetic variance, plus a small part of the epistatic deviations, Lush *(7)* and Cockerham *(2)*. The Component A consists of the differences between station-years. If there are consistent biases in the scoring of the daughters of one bull, as compared to the scoring of the daughters of another, these biases would affect the Component B, but such biases seem unlikely.

## HERITABILITY AND REPEATABILITY

By using the components (Table 2), estimates of heritability and repeatability can be derived according to procedures used by Dickerson *(4)* and

Craig *et al. (3)*. The repeatability of single ratings of the degree of expression of heat among daughters of the same bull is $\frac{C}{C+E} = 0.26$, while that for single ratings among unrelated animals is $\frac{C+B}{C+B+E} = 0.29$. The heritability of single ratings is $\frac{4B}{B+C+E} = 0.21$, but with only 29 sires and 21 degrees of freedom involved, the fiducial limits for this estimate would probably be quite wide. In three station-years, there were more than one breed tested, so this estimate includes these breed differences. These differences, however, were small, and not significant.

It should be remembered that these estimates of repeatability are based on data in which each cow was observed during only one breeding season, and are within-breeding-season estimates. It is likely that estimates based on data covering a number of breeding seasons per cow would be smaller than those shown here.

## DIFFERENCES IN RATINGS AS ASSOCIATED WITH CONCEPTION

After establishing the facts that there are real differences among heifers in the degree of expression of heat, and that these differences have a heritability of approximately 0.21, one wonders whether or not there is a tendency for those heifers showing strong heat symptoms to be more fertile than those with weak heat symptoms. To provide information on this point, the scores for the heats at which first service occurred were classified according to sires and whether or not conception occurred. Thus, the 546 first services were divided into 58 subclasses, ranging from three to 16 observations each, which was the case for one progeny group with three conceptions and 16 failures. Since the subclass numbers were disproportionate, the method presented by Snedecor *(10)*, of the weighted sums of squares of the mean differences between conceived and failures to conceive, was used to calculate the analyses of variance shown (Table 3). As shown by the mean squares (Table 3), the difference between the averages of the two groups, conceived and failure to conceive, is significant at the 0.01 level of probability. The weighted and unweighted means of the group that conceived

TABLE 3

*Analyses of variance of the ratings for the degree of expression of heat at first and second service*

| Source | First-service data | | | Second-service data | | |
|---|---|---|---|---|---|---|
| | Degrees of freedom | Mean square | Components of variance | Degrees of freedom | Mean square | Components of variance |
| Between-progeny groups | 28 | 1.44[++] | A = 0.050 | 28 | 1.85[+,b] | A = 0.121 |
| Conceived *vs.* failure to conceive | 1 | 5.84[++] | B = 0.020 | 1 | 1.95[+a] | B = 0.008 |
| Interaction | 28 | 0.50 | C = 0.002 | 28 | 0.54 | C = 0.010 |
| Error | 488 | 0.46 | E = 0.46 | 255 | 0.48 | E = 0.48 |

[a+] Significant at the 0.05 level of probability.
[b++] Significant at the 0.01 level of probability.

were 2.55 and 2.56, respectively; whereas, the corresponding means for the group that failed to conceive were 2.35 and 2.34. The weighted mean of the differences between the unweighted means of the subclasses $\frac{(SWD)}{(SW)}$ was 0.22. This difference is about one-third of the error standard deviation.

Also (Table 3), a similar analysis of the second-service data is shown. The mean of the group that conceived on second service is significantly higher than the mean of the group that did not conceive, as is evidenced by the mean squares. The weighted and unweighted means of the group that conceived were 2.48 and 2.46, respectively; whereas, the corresponding means for the group that did not conceive were 2.33 and 2.35. The weighted mean of the unweighted differences between subclass means $\frac{(SWD)}{(SW)}$ was 0.17. In this case the difference is only about one-fourth of the error standard deviation. It should be remembered that for both the first and second service, the rating for heat symptoms was made before any knowledge of conception was available.

The components of variance (shown in Table 3) were derived by equating the sample mean squares to the expected mean squares according to Henderson's *(6)* Method 1. The sum of the Components E and C (Table 2) should be approximately equal to the sums of the B, C, and E components for both first and second service (Table 3). The sums from Tables 2 and 3, respectively, are 0.51, 0.48, and 0.50.

By utilizing the components of variance (Table 3), intraclass correlations among the groups that conceived and failed to conceive can be obtained. Under the restrictions that the heifers are at the same station and are by the same bull, these correlations are 0.04 and 0.02, for the first- and second-service date, respectively. These small intraclass correlations indicate that conception or failure to conceive is only slightly associated with the ratings for the degree of expression of heat.

The within-cow difference between the ratings for conception and failure to conceive was investigated by utilizing those 264 cows in which conception did not occur until the second, or a later, service. The difference between the rating $(X^+)$ at the service on which a cow conceived, and the rating $(X^-)$ at the immediately previous service, which was a failure, was obtained. These differences were analyzed according to Student's Method and the following results were obtained:

$$t = \frac{\overline{X}^+ - \overline{X}^-}{\sqrt{\frac{\sigma_D^2}{N}}} = \frac{0.114}{0.0484} = 2.36^+$$

Even though this difference is significant at the 0.05 level of probability, it is small. It is again evident that the magnitude of the rating for the degree of expression of heat is only slightly associated with conception.

## CORRELATION BETWEEN CONCEPTION AND RATINGS OF EXPRESSION OF HEAT SYMPTOMS

The within-progeny groups correlations between the degree of expression of heat symptoms *(x)* and conception *(y)* were 0.154 and 0.129, for first- and second-service data, respectively (Table 4). These correlations are based on 516 and 283 degrees of freedom, respectively, and are significant at the 0.01 and 0.05 levels of probability. These correlations include both the genetic and environmental components of correlation.

## HERITABILITY OF CONCEPTION

In determining the heritability of conception, the data on first and second services were analyzed separately. In explaining the method of analysis, consider the data on the first service only. If a cow conceived on first service, she was given a score of 1, and if she failed to conceive she was given a score of 0. An analysis of variance of these scores is shown (Table 5). It was necessary to determine the difference between progeny groups on a within-station-year basis, since those progeny groups at different stations, and at the same station but in different years, were mated to different sires, and these service-sire differences should not be confounded with the hereditary differences among the sires of the progeny groups. Those progeny groups at the same station in the same year were serviced by artificial insemination with semen from the bulls at one artificial insemination center, and these matings should be largely at random, such that there would not be a serious confounding of service-sire differences with sire differences.

The components of variance (Table 5) were derived by equating the expected mean squares to the sample mean squares according to Henderson's *(6)* Method 1. By using these components, the heritability of conception is 0.040 on the basis of first-service data and — 0.037 on the basis of the second-service data; but, since the differences between progeny groups in the same station-year were not significant, these estimates of heritability should not be considered as being significant. In three station-years there were more than one breed tested, but these differences between breeds were small and not significant. The present estimates of heritability are in agreement with those found by Dunbar and

TABLE 4

*Within progeny group and station-year variances of and correlations between the expression of heat and conception*

| | First-service data | | | | Second-service data | | | |
|---|---|---|---|---|---|---|---|---|
| | Correlation | Degrees of freedom | $\sigma_x^2$ Expression of Heat | $\sigma_y^2$ Conception | Correlation | Degrees of freedom | $\sigma_x^2$ Expression of Heat | $\sigma_y^2$ Conception |
| Within progeny groups | 0.154++[a] | 516 | 0.478 | 0.235 | 0.129+[b] | 283 | 0.462 | 0.232 |
| Within-station-years | 0.151++ | 537 | 0.465 | 0.236 | 0.080 | 304 | 0.500 | 0.231 |

[a] ++ Significant at the 0.01 level of probability.
[b] + Significant at the 0.05 level of probability.

TABLE 5

*Analysis of variance of data on conception at the first and second service*

| Source | First-service data | | | Second-service data | | |
|---|---|---|---|---|---|---|
| | Degrees of freedom | Mean square | Components of variance | Degrees of freedom | Mean square | Components of variance |
| Station-years | 7 | 0.60+[a] | | 7 | 0.74++[b] | |
| Progeny groups in the same station-years | 21 | 0.28 | (0.0025) | 21 | 0.21 | −(0.0022) |
| Error | 517 | 0.23 | (0.2345) | 284 | 0.23 | (0.2327) |

[a]+ Significant at the 0.05 level of probability.
[b]++ Significant at the 0.01 level of probability.

Henderson *(5)*, Carmon *(1)*, and Pou *et al. (9)*. Since the two estimates of heritability bracket zero, and their fiducial limits would probably include zero, it is likely that the heritability of conception is approximately zero.

## DISCUSSION

The correlation between the degree of expression of heat and the conception rate will probably be affected by the experience and keenness of the observer. It is only in the well-managed herds that the observations on heat are comparable to those at the progeny-testing stations. One could argue that strong expressions of heat would be more important in poorly managed herds, in which the weaker expressions of heat would more likely go undetected, than they would be in well-managed herds.

The conception rate at the progeny-testing stations is considerably lower than that in ordinary herds. In the present data, the conception rates were 40.1, 38.3, 37.1, and 30.2% at first, second, third, and fourth services, respectively. Such conception rates are typical for the progeny-testing stations in general. The reasons for the low fertility at the progeny-testing stations, as compared to ordinary herds, are unknown. The high level of feeding and management, and the fact that the heifers are brought together from nearly as many farms as there are heifers at the stations, thus enhancing the risk of transmission of genital infections, may possibly be contributing causes to the low fertility. The low fertility has made it possible to make more observations on the degree of expression of heat on the individual cows than would otherwise have been possible. The relationship between heat score and conception could have been affected by this low level of fertility, but such an effect, if present, would probably be small.

The rating of the degree of expression of heat would seemingly have its most important use as an index in selecting for more fertile cows. If conception at first service is used as a measure of fertility, the regression of the genetic value (additive hereditary value) for conception at first service on the expression of heat is a measure of the value of the latter as an index for increasing the fertility of cattle.

The regression of the genetic value for conception at first service on the expression of heat is $g_x r_{G_x G_y} \frac{g_y \sigma_y}{\sigma_x}$. The symbol $g_x$ is the square root of the herita-

bility of the expression of heat; $r_{G_x G_y}$ is the correlation between genetic values for conception at first service and expression of heat; $g_y$ is the square root of the heritability of conception at first service, $\sigma_y$ is the standard deviation for conception, and $\sigma_x$ is the standard deviation of the expression of heat. By using parameters derived from the first-service data on conception and the estimate of heritability of the expression of heat, $g_x r_{G_x G_y} \frac{g_y \sigma_y}{\sigma_x} = 0.065\ r_{G_x G_y}$. If $r_{G_x G_y} = 1$, then the regression of the genetic value for conception of the expression of heat is 0.065, and if $r_{G_x G_y} = \frac{1}{2}$, then the regression is 0.033, or it is 0.016 if $r_{G_x G_y} = \frac{1}{4}$.

If, on a basis of expression of heat, a breeder picks out the top half of his cows, for conception he could expect these cows to be only 0.035 of a unit better genetically than his herd average, and this would be assuming that $r_{G_x G_y}$ is 1. If the genetic correlation is 0.50, then the genetic gain is 0.018, and only 0.009 if the genetic correlation is 0.25. If the parameters from conception at second service are used, the expected genetic changes involve imaginary numbers, are negative and smaller. From these figures, it can be seen that selection for conception on a basis of expression of heat is feeble. Not only would selection for production be dulled, but there would be practically no gain in conception rate as a result of selecting for degree of expression of heat.

## SUMMARY AND CONCLUSIONS

The degree of expression of heat symptoms was scored on 554 first-calf heifers sired by 29 different sires and distributed among six progeny-testing stations. These scores ranged from 1 for faint expression of heat to 4 for strong expression of heat. The heritability of the expression of heat was found to be 0.21. Those heifers that conceived at first service had a significantly higher rating for expression of heat than those that failed to conceive, the difference being 0.22 unit. Those that conceived at second service had a significantly higher rating than those that failed to conceive, the difference being 0.17 unit.

By scoring the cows 0 for failure to conceive, or 1 for conceiving, the heritability of conception was found to be 0.040 and —0.037, for first and second services, respectively. These estimates are small, and they bracket zero; consequently, the heritability of conception is probably small or zero.

The within-progeny-group correlations between conception and the expression of heat were 0.154 and 0.129 for the first and second service data, respectively. On a basis of first-service data, the regression of the genetic value for conception on the expression of heat was 0.065 $r_{G_x G_y}$, where $r_{G_x G_y}$ is the correlation between the genetic values for conception and expression of heat.

It is concluded that ratings for the degree of expression of heat are of little or no value in selecting for an increased conception rate.

## REFERENCES

(1) CARMON, G. M. Interrelations of Milk Production and Breeding Efficiency in Dairy Cows. *J. Animal Sci.*, **14**: 753. 1955.

(2) COCKERHAM, C. CLARK. An Extension of the Concept of Partitioning Hereditary Variance for the Analysis of Covariance Among Relatives when Epistasis Is Present. *Genetics*, **39**: 859. 1954.

(3) CRAIG, J. V., NORTON, H. W., AND TERRILL, S. W. A Genetic Study of Weight at Five Ages in Hampshire Swine. *J. Animal Sci.*, **15**: 242. 1956.

(4) DICKERSON, G. E. Experimental Design for Testing Inbred Lines of Swine. *J. Animal Sci.*, **1**: 326. 1942.

(5) DUNBAR, R. S., JR., AND HENDERSON, C. R. Heritability of Fertility in Dairy Cattle. *J. Dairy Sci.*, **36**: 1063. 1953.

(6) HENDERSON, C. R. Estimation of Variance and Covariance Components. *Biometrics*, **9**: 226. 1953.

(7) LUSH, J. L. Heritability of Quantitative Characters in Farm Animals. Proc. *VIIIth Intern. Congr. Genetics*. pp. 356-375. 1948.

(8) OLSSON, BÖRJE. Seminföreningarnas Hålkortsbokföring. 1957.

(9) POU, J. W., HENDERSON, C. R., ASDELL, S. A., SYKES, J. F., AND JONES, R. C. A Study of the Inheritance of Breeding Efficiency in the Beltsville Dairy Herd. *J. Dairy Sci.*, **36**: 909. 1953.

(10) SNEDECOR, G. W. *Statistical Methods*. 5th ed. Iowa State College Press, Ames. 1956.

(11) WEBER, E. Untersuchungen über die Brunst des Rindes. *Arch. wiss. prak. Tierheilk.*, **73**: 382. 1911.

# EFFECT OF GRASS SILAGE ON MILK FLAVORS AND BLOOD AND MILK ACETONE BODIES[1, 2]

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**The relationships of acetone bodies in grass silage to levels of blood and milk acetone bodies and to milk flavors were investigated with ten Holsteins for 5 mo. An interrelationship existed between the levels of acetone bodies in silage, blood, and milk. Silage acetone bodies increased in January and February as compared to the period from December to April. There was no relationship between the levels of acetone bodies in individual cows' milk and the flavor of the milk. Feeding grass silage 2 hr. before milking caused off-flavors to appear in the milk.** *Editor.*

Considerable information has been published relative to the effects of silage feeding on milk flavors, and on the time interval between feeding and milking as related to intensity of off-flavors in the milk. However, little is known about the compounds involved which produce the undesirable flavors in milk when silage is fed. Acetone bodies in milk have been associated with cowy, feed, or sweet flavors *(3, 6)*. Certain investigators observed that normal milk contained from 0.53 to 1.80 mg. of acetone bodies per 100 ml. *(2, 5)*, while others have reported none present *(3, 7)*. It has been noted that the level of blood acetone bodies rises 3 hr. after feeding, especially when aged grass silage is fed *(4)*. The same investigators suggested that the increase in blood acetone bodies was due primarily to the feeding of grass silage. Duncan *et al. (2)* summarized the normal levels of blood ketone bodies as observed by numerous investigators.

The present study was initiated to determine the possible relationship of the acetone bodies in grass silage to blood and milk acetone body levels of, and to the flavors of milk produced by, cows fed the silage. Preliminary observations had indicated that these factors might be associated with certain periods of off-flavor difficulties during midwinter.

## EXPERIMENTAL PROCEDURE

Ths study was conducted during a 5-mo. winter feeding period. In the first trial (December, 1955, through March, 1956), ten Holstein cows were paired into two groups on the basis of milk production, body weight, and stage of lactation. They were housed in a well-ventilated, bank-type stanchion barn and were milked and handled uniformly. All cows were turned out daily for exercise. The

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[3] Present address: U. S. Army.

same feeds were offered to each group, consisting of good-quality mixed hay fed ad libitum, concentrates fed according to production at a rate of approximately 1 lb. per 3 lb. of milk, and alfalfa-grass silage which had been preserved with sodium metabisulfite. Silage was fed at the rate of 50 lb. per cow daily, in two equal feedings during the first half of the trial. At the midpoint of the trial, three cows refused a part of their allowance; subsequently, the amount fed was reduced to 40 lb. per cow daily. The five cows designated as controls were fed silage following milking; whereas, silage was fed to the experimental group immediately before the A.M. milking and 2 hr. prior to the P.M. milking.

Silage, blood, and milk samples were obtained at biweekly intervals. Blood samples were drawn from the jugular vein into tubes containing potassium oxalate as an anticoagulant. The blood samples were obtained at approximately 10 A.M. Milk samples were collected at the 5 A.M. milking on the day following blood collection. Silage samples were collected when fed to the cows on both of the above days for each biweekly collecting period. All samples were analyzed for acetone body concentration by the Behre salicylaldehyde method *(1)*. The silage samples were prepared for determination by combining a 1:5 ratio by weight of silage and distilled water. This mixture was shaken vigorously at intervals and allowed to soak for from 18 to 24 hr. The liquid then was decanted, deproteinized with barium hydroxide and zinc sulfate, and the determination run on the filtrate. Milk and blood samples were deproteinized shortly following collection. A person qualified in flavor-judging analyzed the milk samples for the type and intensity of the flavors present in the milk.

A second trial was conducted over a 3-wk. period immediately following the first phase, and utilized three of the original five pairs of cows. Milk samples were collected four times weekly, on Monday P.M., Tuesday A.M., Friday P.M., and Saturday A.M. Blood and silage samples were obtained on Monday P.M. Since silage feeding procedures remained identical to those of Trial I, P.M. blood and milk samples were collected from the experimental group 2.5 hr. after feeding silage; whereas, collections for the control group were made just prior to evening silage feeding, or 10.5 hr. after morning feeding. The groups were reversed at the midpoint of this trial. Acetone bodies were determined on the blood and silage samples *(1)*, and milk samples were judged for flavor.

## RESULTS AND DISCUSSION

The concentration of acetone bodies in the blood, milk, and silage for each biweekly collecting period in Trial I are reported (Table 1). The silage acetone body values are presented as the mean of the two samples at each collecting period, as there was no significant difference between these values. The values for silage acetone bodies increased considerably during the latter part of January and February, in relation to the other sampling periods. An increase in blood and milk acetone body values accompanied this rise for a part of the 2-mo. period. There appeared to be a closer relationship between changes in silage acetone body levels and those in the milk from the experimental cows, which had been fed prior to milking, than between silage levels and control-cow milk

TABLE 1

*Acetone bodies in blood and milk of cows fed grass silage*

| Sampling date (1955-6) | Acetone body concentration (mg/100 ml.) | | | |
|---|---|---|---|---|
| | Silage (1:5 water extract [a]) | Blood [b] | Milk [c] | |
| | | | Experimental cows | Control |
| December 6 | 0.62 | 1.83 | 4.12 | 4.72 |
| December 20 | 1.94 | 1.72 | 3.99 | 3.97 |
| January 3 | 0.80 | 1.87 | 5.10 | 6.57 |
| January 16 | 3.59 | 2.00 | 9.82 | 7.18 |
| January 31 | 3.65 | 3.72 | 8.99 | 4.46 |
| February 14 | 3.56 | 1.77 | 5.32 | 3.78 |
| February 28 | 2.38 | 1.62 | 5.43 | 6.26 |
| March 13 | 1.75 | 2.01 | 5.23 | 7.91 |
| March 27 | 2.19 | 1.87 | — | — |
| Mean | 2.28 ± 0.28 [d] | 2.05 ± 0.08 | 6.00 ± 0.49 | 5.67 ± 0.38 |

[a] Acetone body concentrations are in terms of the water extract.
[b] Mean values for ten cows sampled at each sampling date.
[c] Mean values for five cows. Experimental cows were fed silage prior to milking, controls fed after milking.
[d] Standard error.

levels. There was considerably more variability in the milk acetone body values than there was in the values for blood acetone bodies.

Analysis of variance of the blood and milk acetone body data revealed no significant difference due to the time when silage was fed (experimental *vs.* control cows); however, the differences between sampling periods were highly significant with respect to both blood and silage levels. The statistical correlation between the silage acetone body levels and the acetone bodies in the blood was highly significant ($r = +.285$ with 89 degrees of freedom). There also was a highly significant correlation of $r = +.504$ between the acetone bodies in silage and the level of acetone bodies in the experimental cows' milk, although this relationship did not exist with respect to control cows' milk. Blood acetone body values and milk acetone body values were statistically correlated at the 5% level.

Flavor data were obtained in Trial I; however, the results will be omitted, as they were quite similar to those obtained in Trial II, which will be presented later in this discussion. There was no relationship between the acetone body values of the milk and the intensity of flavors of this milk.

In Trial II, which followed Trial I for 3 wk. during April, three cows were fed silage 2.5 hr. prior to milking. These were designated as experimental cows and compared to three controls fed after milking. The experimental group had a mean blood acetone value of 2.82 mg. acetone bodies per 100 ml. for three sampling periods, whereas the control group exhibited a mean value of 1.85 mg. This difference was highly significant, as measured by the *t* test. The mean silage acetone body value was 1.98 mg. per 100 ml. of water extract.

Milk samples were collected at six evening and six morning milkings in Trial II. At the midpoint the groups were reversed with respect to the time of feeding silage. All samples were judged for flavor and the results are presented (Tables 2 and 3). Silage fed 2 hr. prior to the P.M. milking produced more and stronger off-flavors in the milk than did the feeding of silage at any other interval as

TABLE 2

*Relation between time of feeding silage to type and intensity of off-flavors present in the milk. Flavors present in P.M. milk in Trial II*

| Cow No. | April 6 | April 9 | April 13 | Cow No. | April 16 | April 20 | April 23 |
|---|---|---|---|---|---|---|---|
| Experimental [a] | | | | Control [b] | | | |
| 895 | Silage [4] | Silage [4] | Silage [4] | 895 | Feed [1] | 0 | 0 |
| 864 | Silage [1] | Silage [4] | Silage [2] | 864 | Feed [1] | Feed [1] | Feed [1] |
| 876 | Silage [1] | Silage [4] | Silage [4] | 876 | Feed [1] | 0 | 0 |
| Control [b] | | | | Experimental [a] | | | |
| 918 | Feed [1] | Feed [2] | Silage [2] | 918 | Silage [4] | Silage [4] | Silage [4] |
| 858 | 0 | 0 | Feed [1] | 858 | Silage [1] | Silage [3] | Silage [2] |
| 862 | Feed [1] | Feed [2] | Feed [2] | 862 | Silage [4] | Silage [3] | Silage [4] |

Key: [0] Acceptable flavor, [1] Very slight intensity, [2] Slight intensity, [3] Medium intensity, [4] Strong intensity.

[a] Fed silage 2 hr. before milking.

[b] Fed silage following milking.

studied in these trials. There did not appear to be any more off-flavors present in the milk, when this limited number of cows was fed this particular silage immediately before milking, than when they were fed after milking.

## DISCUSSION

The research reported in these trials suggests that a relationship existed between the levels of acetone bodies in the silage and those of the blood. It also was observed that the blood acetone bodies increased following the feeding of silage and then declined to a normal level. This observation is in agreement with Knodt *et al. (5)*, although they reported a rise of greater magnitude in blood acetone bodies. The values obtained in this study were 2.82, 2.12, 1.99, and 1.85 mg. % at 2.5, 4, 6, and 10.5 hr., respectively, after feeding the silage. Thus, it appears that the greatest impact of silage feeding on the blood acetone body values occurs from shortly after the feeding of grass silage until 4 hr. later.

There also was evidence to a lesser degree that the level of acetone bodies in silage affects the level of milk acetone bodies. There was no apparent relationship between the level of acetone bodies in the milk and the off-flavors present in the milk. This lack of relationship may be resulting from the measurement of total

TABLE 3

*Flavors present in A.M. milk in Trial II*

| Cow No. | April 7 | April 10 | April 14 | Cow No. | April 17 | April 21 | April 24 |
|---|---|---|---|---|---|---|---|
| Experimental [a] | | | | Control [b] | | | |
| 895 | 0 | 0 | 0 | 895 | 0 | 0 | 0 |
| 864 | 0 | 0 | 0 | 864 | Feed [1] | 0 | 0 |
| 876 | 0 | 0 | 0 | 876 | 0 | 0 | 0 |
| Control [b] | | | | Experimental [a] | | | |
| 918 | Feed [1] | 0 | 0 | 918 | Feed [2] | Feed [1] | Feed [2] |
| 858 | 0 | 0 | 0 | 858 | 0 | 0 | 0 |
| 862 | 0 | Feed [1] | 0 | 862 | Feed [1] | 0 | Feed [1] |

Key: (Same as for Table 2).

[a] Fed silage immediately before milking.

[b] Fed silage following milking.

acetone bodies, instead of just the acetone molecule which is presumably the main flavor component of the acetone bodies.

There seems to be no ready explanation for the rise in acetone bodies in the silage during the months of January and February. Conceivably, it could be the results of an increased bacterial or enzymatic action.

The flavors obtained in relation to the time of feeding silage indicate that a certain amount of time must transpire before the flavor compounds in the silage can reach the milk, or be metabolized from silage components.

## SUMMARY

Acetone bodies were measured in the blood and milk of ten Holstein cows at intervals over a 5-mo. winter feeding period. Acetone body concentrations also were measured in the grass-legume silage which was fed as a part of the cows' ration. Milk produced by the cows was scored for type and intensity of flavor present. An interrelationship existed between the levels of acetone bodies in the silage, blood, and milk. Silage acetone bodies increased considerably in January and February, in relation to the other sampling periods, which extended from December to April. There was no apparent relationship between the levels of acetone bodies in the individual cows' milk and the flavors of the milk, as measured in this study. The most prominent off-flavors in the milk resulted from feeding the grass silage 2 hr. prior to milking.

## ACKNOWLEDGMENT

The authors gratefully acknowledge the services of G. H. Watrous, Jr. and F. J. Doan, who scored all milk samples for flavor.

## REFERENCES

(1) Behre, J. A. A Modified Salicylaldehyde Method for the Determination of Acetone Bodies in Blood and Urine. *J. Biol. Chem.*, **136**: 25. 1940.

(2) Duncan, C. W., Huffman, C. F., and Tobin, H. A. A Chemical Study of Ketosis in a Dairy Herd. *J. Am. Vet. Med. Assoc.*, **95**: 690. 1939.

(3) Josephson, D. V., and Keeney, D. G. Relationship of Acetone Bodies to "Cowy" Flavor in Milk. *Milk Dealer*, **36**(10): 40. 1947.

(4) Knodt, C. B., Shaw, J. C., and White, G. C. Studies on Ketosis in Dairy Cattle. I. Effect of Stall and Pasture Feeding upon the Concentration of Blood and Urinary Acetone Bodies of Dairy Cattle. *J. Dairy Sci.*, **25**: 837. 1942.

(5) Knodt, C. B., Shaw, J. C., and White, G. C. Studies on Ketosis in Dairy Cattle. II. Blood and Urinary Acetone Bodies of Dairy Cattle in Relation to Parturition, Lactation, Gestation, and Breed. *J. Dairy Sci.*, **25**: 851. 1942.

(6) Strobel, D. R., and Babcock, C. J. An Analysis of Flavor Research on Milk. *Milk Dealer*, **43**: (7): 80. 1954.

(7) Thin, C., and Robertson, A. The Estimation of Acetone Bodies. *Biochem. J.*, **51**: 218. 1952.

# CIRCULATION CLEANING[1]

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**The role of turbulence in cleaning, and the relationship of time, temperature, and solution strength to cleaning effectiveness, were studied on test discs, using $P^{32}$-labeled milk to indicate organic and inorganic residues. Reynolds numbers were used to indicate turbulence, which was most important in cleaning, with temperature being less significant. Temperature and turbulence became less important as the effectiveness of the detergent increased.**

*Editor.*

Reports of previous studies on recirculation cleaning are not in close agreement as to the effect of such variables as time and temperature; the influence of velocity has been more clearly indicated, but is yet to be interpreted on the basis of turbulency. The Reynolds number, widely used in hydraulics as a measure of turbulence, would be useful for relating fluid flow to cleaning effectiveness.

This paper presents the results of studies on the role of turbulence in cleaning, and the relationships of time, temperature, and solution strength to cleaning effectiveness.

Evaluation of cleaning procedures has been limited by the difficulty of determining the degree of soil removal. Various workers have used bacteriological methods, colorimetric and visual inspection, or tracer techniques, each of which possesses disadvantages. Visual inspection is generally conceded to be unreliable for quantitative estimation, and modifications involving the use of dyes or fluorescent materials have not proved satisfactory. A bacteriological count obtained by swab test does not necessarily correlate with cleanliness, and Holland *et al. (7)* concluded that bacteria counts could not be recommended as the sole measure of cleanliness. Sieberling and Harper *(13)* found that soil deposits could be measured with greater sensitivity by radioactive tracer techniques than by bacteriological examination. They reported that $P^{32}$ was not a satisfactory tracer for this purpose, for it reacted irreversibly with stainless steel, but that $Ca^{45}$ seemed to yield reliable results. Cucci *(2)* utilized $P^{32}$ in studying milk deposits on rubber, pyrex, and tygon tubing, but under rather unnatural conditions.

## METHODS AND PROCEDURE

A constant-head recirculating tower, available from earlier investigations on cleaning and corrosion, was used for all runs except determinations of correlating data for the turbulence variable. This apparatus, constructed of glass, ce-

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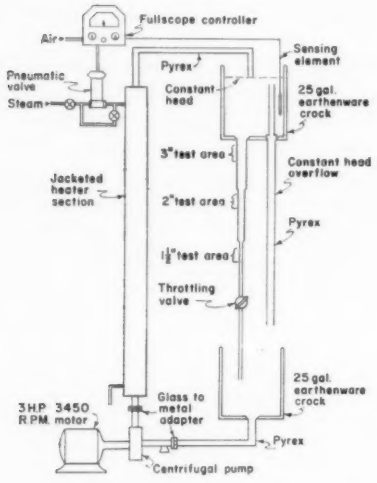


FIG. 1. Recirculating tower.

ramics, and neoprene, permitted accurate velocity control and evaluation of three flow conditions simultaneously (Figure 1).

To investigate the applicability of results to commercial practice, a special pipe section was constructed of 1½ o.d. stainless-steel tubing. This device, used only to study and correlate the influence of turbulency, was operated in a horizontal position (Figure 2).

Preliminary studies indicated that the amount of soil on a test disc could be accurately estimated with radioactive tracers. Because it is less difficult to determine low concentrations of $P^{32}$ than of $Ca^{45}$ (maximum $\beta$ particle energies 1.7 and 0.254 Mev, respectively), there seemed to be a distinct advantage in using $P^{32}$. Exploratory runs indicated that 99% of $P^{32}$-labeled milk films were

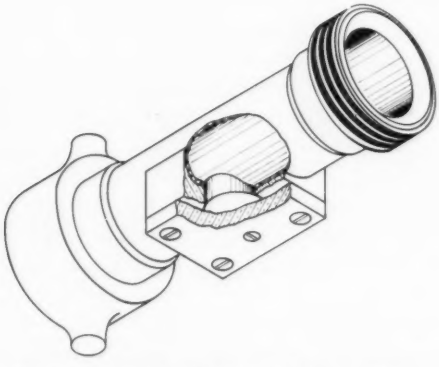

FIG. 2. Diagonal section of pipe test section.

removed by adequate cleaning, and it was concluded that $P^{32}$ was a satisfactory tracer for these experiments. Counting was done with a thin-window Geiger tube and a Nuclear 163 scaler. To permit a more critical study, procedures were adopted for *(a)* preparing uniform and standard soil films, and *(b)* accurately measuring the amount of soil on a surface. The authors concluded that test strips would facilitate the preparation of films that would be more nearly uniform from one trial to another. The test strips were of 18-8 stainless steel 15/16-inch in diameter, flat circular discs in the recirculating tower, and machined inserts in the sanitary pipe test section. These fitted directly into the counting cups of the scaler.

*Preparation of milk films.* A simple device was constructed for drying films of radioactive milk on test discs. Test discs were pushed about ¼-inch into the large ends of rubber bell-reducers, used to eliminate splash in suction aspirators (Fisher Splashgon), thereby forming miniature vats. The small ends of the casings were slipped over the end of short ⅜-inch copper tubes, soldered erect to holes regularly spaced near the edge of the top of a No. 10 can from which the bottom was removed. Steam was applied at this point for drying the discs.

Approximately 100 $\mu$c. of a radioactive phosphorous solution was added to 100 ml. of homogenized milk; from 0.5 to 1.0 ml. of this labeled milk was placed on each disc. In the developmental phase, steam was applied until the discs approached dryness, when the excess milk was decanted. But results proved more reproducible if the discs were steamed to dryness, so this procedure was adopted.

## RESULTS AND DISCUSSION

*Comparison of in vivo and in vitro labeled milk.* In most work of this type, the tracer is an inorganic salt, assumed to provide a suitable index of both organic and inorganic residues. Ionization and diffusion of inorganic tracers, however, could cause a disproportionate decrease in radioactivity, rendering them ineffective as an index of organic residues.

To investigate this possibility, a sample of in vivo labeled milk was prepared by injecting a cow intravenously with 18 mc. of $P^{32}$ solution and collecting milk three hours later. This method has been described in detail elsewhere *(11)*. Aten and Kevesy *(1)* have shown that casein phosphorus, ester phosphorus, and inorganic phosphorus each have nearly the same $P^{32}$ specific activities by 2¼ hr. after injection.

Test discs prepared with in vivo and in vitro milks were subjected to identical cleaning regimens and the losses in activity determined. Because no significant differences were detected, the authors concluded that the use of in vitro labeled milk was justified as an index of both organic and inorganic residues.

*Effect of turbulency.* The cleaning effect ascribed to velocity is probably due to turbulence. The latter is, of course, a manifestation of the former, if velocity is the only variable in the Reynolds number. However, when such factors as diameter, or diameter and velocity, are changed, the relationship becomes more complicated, and velocity alone cannot be used to describe turbulence.

Consequently, to evaluate the role of turbulency in circulation cleaning, all data were plotted with a Reynolds number as the abscissa. In the pipe test section, this is expressed in the standard form:[2]

$$Re = \frac{4 \, v \, r_h \rho}{\eta} = \frac{v \, D \rho}{\eta}$$

For tests in the recirculation tower, the Reynolds number was defined as if the plate strips divided the flow channel into two parts. Thus

$$Re = \frac{v\left[\dfrac{8(\pi+1)}{\pi D}\right]}{\eta}$$

These runs (Figure 3) were all made at a temperature of 50° C. The data show a sharp break in cleaning effectiveness at Re 25,000. There was little cleaning action at any lower Reynolds number. This is illustrated in Figure 4, which

[2] Symbols: D — diameter (ft.); $r_h$ — hydraulic radius $\frac{\text{cross sectional area flow path (ft.}^2\text{)}}{\text{wetted perimeter ft.}}$; Re — Reynolds number; v — velocity ft/sec.; y — % radioactivity; $\eta$ — viscosity $\frac{\text{lb}_m}{\text{hr-ft.}}$; $\theta$ — time (min.); $\rho$ — density $\frac{\text{lb}_m}{\text{ft}^3}$.

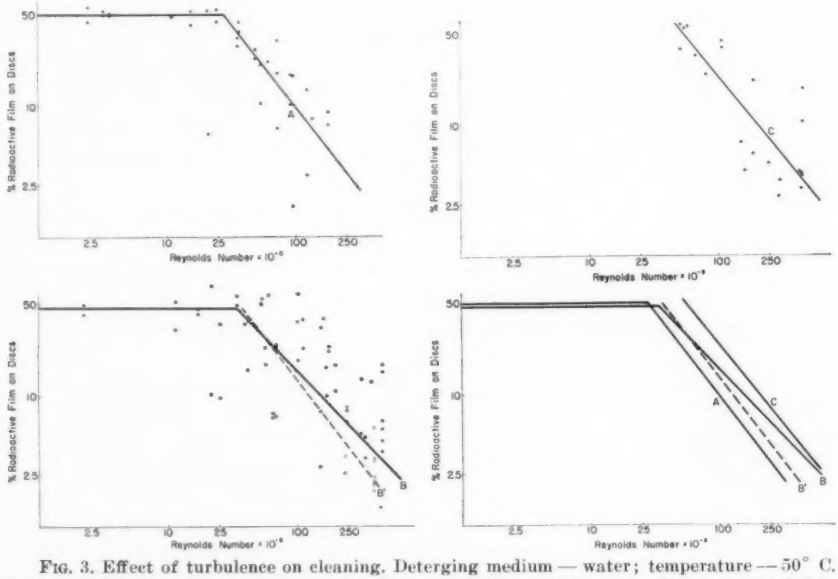


FIG. 3. Effect of turbulence on cleaning. Deterging medium — water; temperature — 50° C. A: Recirculation tower, 10-min. runs. B: Pipe test section, 10-min. runs, all points included in the analysis. B': Pipe test section, 10-min. runs, analysis restricted to points with clear circles and those in zone d. C: Pipe test section, 5-min. runs.

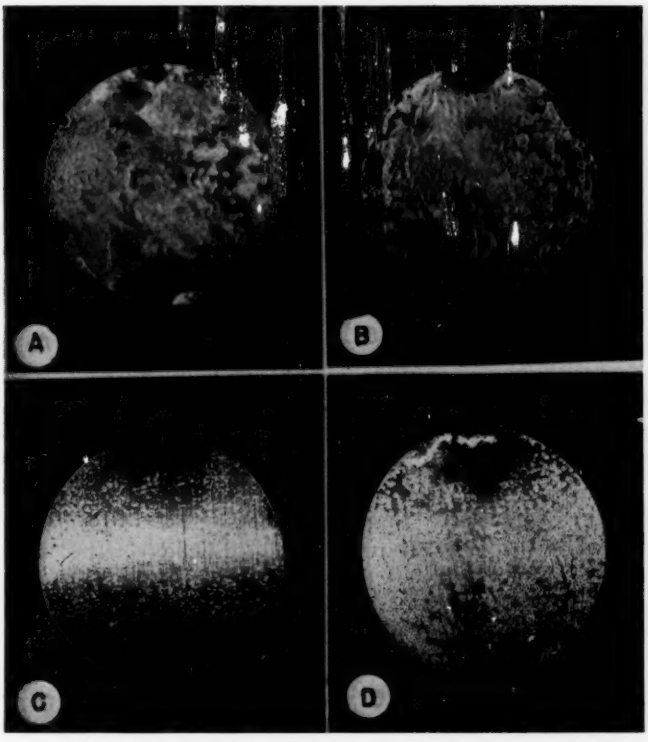


FIG. 4. Pipe section test discs. Deterging medium—water; temperature—50° C. A: Unwashed milk film. B: Washed 10 min. at Re = 50,000. C: Washed 10 min. at Re = 350,000. D: Washed 5 min. at Re = 350,000.

shows (A) an unwashed disc; (B) a disc cleaned for 10 min. at Re 50,000, and (C) a disc cleaned for 10 min. at Re 350,000.

The authors concluded the data were best satisfied by straight lines intersecting at Re 25,000. A statistical analysis was made of all data with Reynolds numbers of 25,000 and above, to determine the regression line and statistical significance. A summary of this analysis is shown (Table 1). All correlations are highly significant.

Careful examination of the last few runs indicated that, at this phase of the work, a residual radioactivity on the edges of the discs caused disproportionately high counts after washing. The radioactivities of these washed discs were determined, the disc surfaces carefully scoured with detergent, and the activities redetermined. The residual radioactivity was treated as background count. Discs handled in this manner are shown as clear circles (Figure 3).

Consequently, the data were analyzed not only with all points used but also using only the points of high radioactivity (Reynolds numbers 34,000 to 60,000)

TABLE 1
*Correlation coefficients for Figure 3*

| | Curve | Correlation coefficient |
|---|---|---|
| Flat discs | A | 0.7 |
| 10 minutes | B | 0.64 |
| | B′ | 0.995 |
| 5 minutes | C | 0.8 |

and the points with clear circles. The former values were chosen because the residual radioactivity of the films themselves would be large compared to any contamination that might have occurred. Result of this analysis is shown as the dashed line (Figure 3, B′).

Comparison of Curves A and B or B′ shows that the data for the recirculating tower correlate quite well with the results obtained in the pipe section. On the basis of these curves, the authors concluded that data obtained with one test section would apply equally to the other.

*Effect of time.* To evaluate the effect of time, a series of duplicate runs in the pipe test section was made in 5 and 10 min. Results are shown in Curves 3B, 3B′, and 3C. In determining the regression line, all points were considered, although some doubt (due to the possibility of contamination) may be attached to the five values that lie above the line. In comparison with the clear-circled values on the 10-min. run, however, the scattering does not seem severe. A visual comparison of the effect of time may be made by consulting Figures 4,C and 4,D.

In any such phenomenon as this, it is reasonable to expect the time factor to be an exponential function. By using curves 3B′ and 4C, this relationship can be expressed as:

$$\frac{y_{10}}{y} = e^{-0.1(10-\theta)}$$

for any given Reynolds number. For adequate cleaning, then, time may be decreased as turbulence is increased.

*Effect of temperature.* A series of runs in the recirculating tower were made to study the effect of temperature on cleaning. The results (shown in Figure 5) indicate that higher temperatures help cleaning, but that turbulence is much more important. Also, higher temperatures seem to be a slightly more important factor in the lower turbulency range.

*Effect of adding plastic beads.* The additional abrasive effect of substances such as plastic beads in suspension might be expected materially to increase cleaning effectiveness. Such additives would have their greatest value in cases of severe burn-on, as in tube heaters. Therefore, test discs prepared in the usual manner were further dried under infrared radiation until some scorching was evident. Approximately 200 g. of Styron beads, 1/16-inch in diameter, were placed in the apparatus and recirculated with the water. The effect on the scorched residues is shown (Figure 6,1), and the effect on residues not subjected to infrared scorching is illustrated (Figure 6,2). It is apparent that abrasion contributed to the cleaning operation.

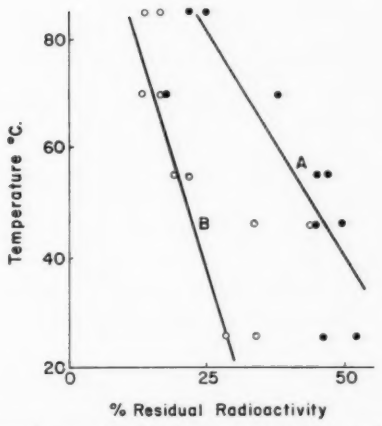


Fig. 5. Effect of temperature on cleaning for two Reynolds numbers. Deterging medium—water; 10-min. runs. A: Re = 36,600. B: Re = 72,000.

*Effect of various cleaner ingredients.* The effectiveness of alkaline cleaner ingredients (sodium carbonate and sodium metasilicate), a sequestering agent (tetrasodium pyrophosphate), and a wetting agent (Trend 40) was also investigated. The effect of turbulence (Figure 7) or temperature (Figure 8) becomes less important as the physico-chemical effectiveness of the detergent increases.

## SUMMARY

The relationship between cleaning effectiveness and variables such as temperature, turbulence, time, and detergent fraction was studied on test discs.

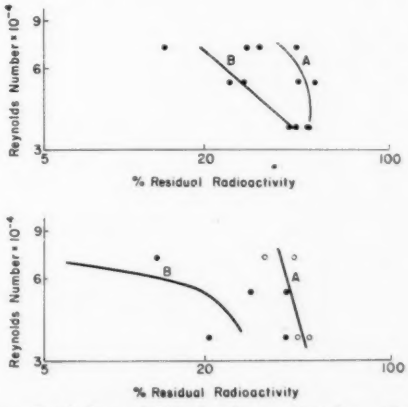


Fig. 6. Effect of recirculating plastic beads on cleaning. Deterging medium—water; temperature—46° C; 10-min runs. 1—Milk residues applied with steam. 2—Milk residues applied with steam and thoroughly dried with infrared radiation. A—Standard washing procedure, no beads. B—Plastic beads added.

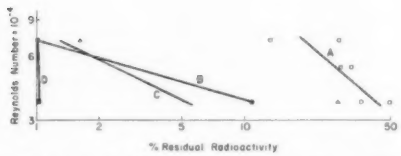


FIG. 7. Comparison of detergent additives on cleaning effectiveness for different degrees of turbulence. A: Water, 10-min. run. B: 0.1% sodium metasilicate, 5-min. run. C: 0.06% sodium metasilicate and 0.04% tetrasodium pyrophosphate, 5-min. run. D: 0.04% sodium metasilicate, 0.03% tetrasodium pyrophosphate, 0.02% sodium carbonate, and 0.01% trend 40, 5-min. run.

Cleaning effectiveness was evaluated by using $P^{32}$-labeled milk. It was determined that in vitro additions of radioactive phosphorus serve as indices of both organic and inorganic milk residues.

Because the relationship of fluid flow to cleaning effectiveness is probably a function of turbulency, the data concerned with fluid flow were calculated on the basis of Reynolds numbers. The abrasive effect of a suspension of plastic beads was found to contribute to cleaning, particularly in cases of burn-on. The results of this study indicate that turbulency is of primary importance to cleaning, with temperature less significant. As the physico-chemical effectiveness of the deterging medium is increased, the effect of temperature and turbulence becomes less pronounced.

ACKNOWLEDGMENTS

The recirculating tower was constructed by J. Erway[3] and G. L. Gallaher[4] to study cleaning and corrosion. Some of the materials of construction were donated by the Pacific Coast office of

[3] Present address, Joliet, Illinois.

[4] Present address, Mechanicsville, Iowa.

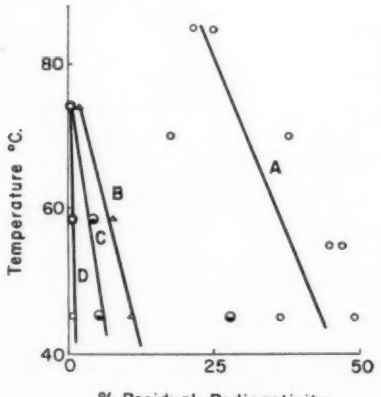


FIG. 8. Comparison of detergent additives on cleaning effectiveness for several temperatures. Re = 72,000. A: Water, 10-min. run. B: 0.1% sodium metasilicate, 5-min. run. C: 0.06% sodium metasilicate and 0.04% tetrasodium pyrophosphate, 5-min. run. D: 0.04% sodium metasilicate, 0.03% tetrasodium pyrophosphate, 0.02% sodium carbonate, and 0.01% Trend 40, 5-min. run.

the Corning Glass Works. The authors made slight modifications in the apparatus to permit high-turbulency studies.

The donation of various detergent fractions by the Continental Chemical Company, Sacramento, California, is gratefully acknowledged.

The authors wish to acknowledge the technical assistance of H. Rapp[5] on the earlier experimental runs.

[5] Present address, *Food Engineering*, New York, N. Y.

## REFERENCES

(1) Aten, A. H. W., Jr., and Kevesy, G. Formation of Milk. *Nature*, **142**: 111. 1938.

(2) Cucci, M. W. Use of Radioactive Phosphorus to Measure the Amount of Milkstone Deposited on Rubber, Pyrex Glass, and Tygon Tubings. *J. Milk and Food Tech.*, **17**: 332. 1954.

(3) Fleischman, F. F., Jr., and Holland, R. F. Permanent Pipelines Cut Cleaning Costs. *Food Eng.*, **23**, 11: 58. 1951.

(4) Fleischman, F. F., Jr., White, J. C., and Holland, R. F. Glass Lines Do A-1 Job, No "Take Down" to Clean. *Food Inds.*, **22**: 1686. 1950.

(5) Fortney, C. G., Jr., Baker, M. P., and Bird, E. W. Cleaning Stainless Steel Sanitary Lines in Place. *J. Milk and Food Tech.*, **18**: 150. 1955.

(6) Holland, R. F. Cleaned-In-Place Pipelines. *Food Eng.*, **26**, 10: 84. 1954.

(7) Holland, R. F., Shaul, V. D., Theokas, D. A., and Windlan, H. M. Cleaning Stainless Lines in Place. *Food Eng.*, **25**, 5: 75. 1953.

(8) Moore, A. V. In-Place-Cleaning of Sanitary Pipelines. *Milk Dealer*, **41**: 146. 1952.

(9) Moore, D. H., Tracy, P. H., and Ordal, Z. V. Permanent Pipelines for Dairy Plants. *J. Dairy Sci.*, **34**: 804. 1951.

(10) Parker, R. B., Elliker, P. R., Nelson, G. T., Richardson, G. A., and Wilster, G. H. Cleaning Pipelines in Place. *Food Eng.*, **25**, 1: 82. 1953.

(11) Ralston, N. P., Kleiber, Max, and Smith, A. H. Venous Catheterization of Dairy Cows. *J. Dairy Sci.*, **32**: 889. 1949.

(12) Sieberling, D. A. Automation in the Cleaning-In-Place Operation. *Am. Milk Rev.*, **17**, 2: 32. 1955.

(13) Sieberling, D. A., and Harper, W. V. Evaluation of the Cleanability of C.I.P. Automatic Valves. Paper M-46, presented at the 51st Annual Meeting, A.D.S.A. 1956.

(14) White, J. C. Permanent Pipelines Today and Tomorrow. *Am. Milk Rev.*, **16**, 10: 48. 1954.

# EFFECT OF MILKING TIME ON SOLIDS IN MILK FROM HOLSTEINS[1]

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**Variations in the solids between mornings' and evenings' milk of 40 Holsteins were studied for two consecutive milkings during three years. There were significant differences among cows for pounds of milk, fat, solids-not-fat and total solids and for their percentages in milk. Significantly more milk was produced in the morning and there were significant differences in the amount of solids in mornings' and evenings' milk.** *Editor.*

In recent years, the dairy industry has placed increasing emphasis on the nonfat solids portion of milk, and attention has been focused on the scarcity of comprehensive data illustrating the characteristics of solids in milk. This study was conducted for the purpose of determining variations of the solid-milk components, between the morning and evening milk of individual cows.

Whether milk from an individual cow is sampled during the morning or evening milking may have an effect on its solids content. Citations of Peterson *(5)*, Turner *(8)*, and Wing *(9)*, on milk yield and fat test of morning and evening milk, have led to the conclusion that when the interval was even, there was a greater milk yield but a lower fat test in the morning milk. Bailey *(1)* and Bartlett *(2)* showed a very slight decline in solids-not-fat of morning herd milk over evening herd milk.

Eckles and Shaw *(3)*, reporting on the average production of five cows for 14 consecutive days, showed that the solids-not-fat content was higher in the evening milk for two Holsteins, but lower in the evening milk for one Ayrshire and two Jersey cows. Sprecht *et al. (7)* found no significant difference in the morning and evening milk from 12 individual Jersey cows, milked regularly with an 11½-hr. night-time interval. However, they found a significant difference in favor of the morning milk yield.

## EXPERIMENTAL PROCEDURE

Milk for this study was obtained from the University of Wyoming purebred Holstein-Friesian herd. Samples were collected monthly from individual cows during two consecutive milkings. The herd was milked regularly with a 12-hr. interval between morning and evening milking times. This interval would sometimes vary by 30 min. Milk from 40 cows was analyzed over a period of three years, to discount effects of all factors except those being studied. The following determinations were made on milk samples from individual cows: fat content

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by the Babcock method; total solids by the Dietert method *(4)*, using the Dietert speed oven for drying the milk samples, and solids-not-fat by difference between total solids and fat. Data were tested statistically by the analysis of variance techniques as outlined by Snedecor *(6)*.

## RESULTS AND DISCUSSION

Forty cows were tested for the following factors of milk production: milk yield, butterfat yield, solids-not-fat yield, total solids yield, butterfat percentage, solids-not-fat percentage, and total solids percentage. There was an average of 10.1 observations per cow.

The mean yields per cow for each milking, morning and evening, are shown (Table I). The over-all milking-time means for yield also are included in the table.

*Milk yield.* When the differences among cows for milk yield were tested according to the analysis of variance *(6)*, cow differences were significant at the 1% level. The mean morning and evening milk yields were 18.56 and 16.47 lb., respectively; milking-time differences also were significant at the 1% level, with an "F" value of 23.57. No evidence was found for interaction between cows and milking time.

The difference between morning and evening milkings is in agreement with previous reports *(5, 7-9)*. With a slight difference in the intervals between milkings, an even greater difference in milk yield between milkings would be expected. Wing *(9)* reported differences in milk yield even when the intervals between milkings were equal.

*Butterfat yield.* Differences among cows for butterfat yield were highly significant. Butterfat-yield milking-time means were 0.68 lb. for morning milk and 0.62 lb. for evening milk. Significant differences at the 1% level were found between milking times, with an "F" value of 11.86. Evidence of an interaction between cows and milking time for butterfat yield was not found.

Butterfat yield followed closely the pattern found for milk yield. This should be expected, except that there have been reports of a higher butterfat percentage in the evening's milk yield *(9)*. A lower butterfat percentage in the morning's milk yield might tend to reduce differences in butterfat yield, owing to the milk-yield differences. However, the reduced butterfat percentage did not offset the differences in yield.

*S.N.F. yield.* The yield of solids-not-fat was found to be highly significantly different among cows. An "F" value of 17.85 was significant at the 1% level between milking times. Solids-not-fat yield means for morning and evening milk were 1.60 and 1.42 lb., respectively. Interaction between milking time and cows was not found.

There have been very few reports concerning the solids-not-fat portion of individual cows in respect to morning and evening milkings. Although Eckles and Shaw *(3)* reported a higher solids-not-fat yield for two Holsteins, not one of the 40 cows in this study had a higher solids-not-fat yield for the evening milking (see Table 1).

TABLE 1

*Average yields of milk components, by individual cows and time of milking*

| Cow No. | Months of observations | Milk | | Butterfat | | Solids-not-fat | | Total solids | |
|---|---|---|---|---|---|---|---|---|---|
| | | Morning | Evening | Morning | Evening | Morning | Evening | Morning | Evening |
| | | *(lb.)* | | | | | | | |
| 1 | 12 | 20.31 | 18.85 | 0.73 | 0.65 | 1.77 | 1.62 | 2.50 | 2.27 |
| 2 | 10 | 18.08 | 17.06 | 0.66 | 0.62 | 1.59 | 1.48 | 2.25 | 2.10 |
| 3 | 8 | 21.41 | 19.94 | 0.83 | 0.74 | 1.86 | 1.70 | 2.69 | 2.44 |
| 4 | 9 | 16.64 | 14.14 | 0.60 | 0.53 | 1.46 | 1.28 | 2.06 | 1.81 |
| 5 | 10 | 19.08 | 15.89 | 0.64 | 0.60 | 1.61 | 1.35 | 2.25 | 1.95 |
| 6 | 8 | 17.19 | 15.25 | 0.64 | 0.59 | 1.45 | 1.31 | 2.09 | 1.90 |
| 7 | 17 | 22.30 | 19.83 | 0.77 | 0.71 | 1.89 | 1.66 | 2.66 | 2.37 |
| 8 | 10 | 21.10 | 18.53 | 0.79 | 0.66 | 1.81 | 1.57 | 2.59 | 2.23 |
| 9 | 18 | 16.84 | 15.22 | 0.61 | 0.51 | 1.42 | 1.28 | 2.03 | 1.79 |
| 10 | 9 | 19.20 | 17.90 | 0.65 | 0.64 | 1.57 | 1.48 | 2.22 | 2.12 |
| 11 | 11 | 19.05 | 15.31 | 0.79 | 0.58 | 1.68 | 1.35 | 2.46 | 1.93 |
| 12 | 11 | 23.92 | 21.37 | 0.90 | 0.89 | 2.08 | 1.86 | 2.98 | 2.75 |
| 13 | 6 | 21.67 | 18.90 | 0.69 | 0.67 | 1.89 | 1.60 | 2.58 | 2.27 |
| 14 | 12 | 23.65 | 20.92 | 0.76 | 0.71 | 2.03 | 1.77 | 2.79 | 2.48 |
| 15 | 9 | 24.92 | 23.96 | 0.87 | 0.95 | 2.24 | 2.13 | 3.11 | 3.08 |
| 16 | 10 | 23.35 | 20.36 | 0.81 | 0.68 | 2.02 | 1.72 | 2.83 | 2.39 |
| 17 | 3 | 13.03 | 10.13 | 0.55 | 0.45 | 1.15 | 0.92 | 1.70 | 1.37 |
| 18 | 11 | 20.29 | 17.20 | 0.78 | 0.63 | 1.79 | 1.49 | 2.56 | 2.12 |
| 19 | 10 | 16.47 | 15.43 | 0.72 | 0.61 | 1.53 | 1.39 | 2.25 | 2.00 |
| 20 | 12 | 13.98 | 13.61 | 0.56 | 0.57 | 1.20 | 1.17 | 1.77 | 1.73 |
| 21 | 13 | 16.85 | 14.51 | 0.59 | 0.63 | 1.33 | 1.15 | 1.92 | 1.78 |
| 22 | 10 | 20.11 | 17.80 | 0.69 | 0.60 | 1.63 | 1.44 | 2.32 | 2.04 |
| 23 | 9 | 17.20 | 14.79 | 0.67 | 0.61 | 1.49 | 1.25 | 2.17 | 1.86 |
| 24 | 8 | 14.11 | 12.54 | 0.53 | 0.50 | 1.10 | 1.10 | 1.73 | 1.60 |
| 25 | 8 | 20.46 | 17.14 | 0.77 | 0.67 | 1.79 | 1.52 | 2.55 | 2.19 |
| 26 | 9 | 16.73 | 14.97 | 0.58 | 0.54 | 1.43 | 1.28 | 2.01 | 1.82 |
| 27 | 9 | 19.61 | 16.78 | 0.68 | 0.61 | 1.75 | 1.50 | 2.43 | 2.11 |
| 28 | 10 | 14.06 | 11.71 | 0.46 | 0.44 | 1.09 | 0.98 | 1.55 | 1.42 |
| 29 | 9 | 17.41 | 15.06 | 0.61 | 0.54 | 1.45 | 1.36 | 2.06 | 1.90 |
| 30 | 10 | 21.72 | 20.25 | 0.92 | 0.91 | 1.92 | 1.75 | 2.84 | 2.66 |
| 31 | 11 | 15.75 | 15.25 | 0.65 | 0.68 | 1.40 | 1.37 | 2.05 | 2.05 |
| 32 | 11 | 16.70 | 13.89 | 0.63 | 0.50 | 1.41 | 1.20 | 2.04 | 1.70 |
| 33 | 12 | 14.92 | 13.35 | 0.68 | 0.58 | 1.34 | 1.23 | 2.03 | 1.81 |
| 34 | 12 | 23.78 | 20.62 | 0.73 | 0.73 | 1.99 | 1.70 | 2.73 | 2.43 |
| 35 | 8 | 9.69 | 8.81 | 0.38 | 0.34 | 0.91 | 0.82 | 1.29 | 1.16 |
| 36 | 9 | 20.11 | 18.27 | 0.75 | 0.63 | 1.77 | 1.57 | 2.52 | 2.20 |
| 37 | 9 | 15.73 | 14.01 | 0.53 | 0.51 | 1.35 | 1.19 | 1.88 | 1.70 |
| 38 | 12 | 17.25 | 15.32 | 0.57 | 0.54 | 1.48 | 1.32 | 2.05 | 1.87 |
| 39 | 12 | 14.64 | 13.14 | 0.59 | 0.54 | 1.22 | 1.10 | 1.81 | 1.63 |
| 40 | 8 | 15.14 | 12.38 | 0.58 | 0.46 | 1.34 | 1.10 | 1.91 | 1.55 |
| Mean | 10.1 | 18.56 | 16.47 | 0.68 | 0.62 | 1.60 | 1.42 | 2.28 | 2.03 |

*Total solids yield.* Cow differences in total solids yield were significant at the 1% level. The mean morning and evening total solids yields were 2.28 and 2.03 lb., respectively. The "F" value of 20.88 for milking time was significant at the 1% level. The interaction between cows and milking time was not significant. With higher yields of butterfat and solids-not-fat in the morning milk yield, the yield in total solids must be greater in the morning milk. Thus, butterfat, solids-not-fat, and total solids yields have paralleled milk yield.

*Butterfat percentage.* When tested by the analysis of variance *(6)*, differences among cows for butterfat percentage were found to be highly significant. The over-all milking-time butterfat-percentage weighted means were 3.66% for

morning milk and 3.76% for evening milk. The differences in butterfat percentages for milking times were significant at the 5% level, with an "F" value of 4.51. No evidence was found of an interaction between cows and milking time for butterfat percentage.

The results found here for butterfat percentage agree with those reported by Wing *(9)*. With the morning milk yield higher than the evening milk yield, the butterfat percentage was less than in the evening milk yield.

*Solids-not-fat percentage.* The differences between cows for solids-not-fat percentage were significant at the 1% level. An "F" value of 0.88 for milking times did not approach the 5% level of significance. The morning and evening solids-not-fat percentage averages were 8.60 and 8.59%, respectively. No evidence for an interaction between cows and milking time was found.

Finding no difference in solids-not-fat percentage between morning and evening milk yields indicates a complete departure from the trends found in the other phases of this study. The differences in solids-not-fat yield that were found are then due to the differences found in milk yield alone.

*Total solids percentage.* Total solids percentage paralleled butterfat percentage closely. Differences among cows for total solids percentage were highly significant. The means for total solids percentage were 12.26 and 12.36%, for morning and evening milking times, respectively. Milking-time differences were significant at the 5% level, having an "F" value of 4.00. As in all previous analysis in this study, no interaction was found between cows and milking time.

These differences in percentage of total solids are the result of the differences in butterfat percentage. With a rise in butterfat percentage and no change in solids-not-fat percentage in the evening milk yield compared to the morning milk yield, a paralleled rise in total solids, therefore, is obtained.

This study further indicates the importance of milk yield as opposed to butterfat, solids-not-fat, and total solids percentages. Increased percentage of the solid components of Holstein-Friesian cows' milk with a decreased milk yield does not balance the effect of decreased yield. Actual yield of the solid components of milk depends primarily upon the quantity of milk yield, as indicated in this study. Apparently, there are no cow differences in this respect, since all phases of interaction between cows and solid components were not significant.

Finding highly significant differences among cows for each milk-solid component indicates that further study of the components is required on an individual basis. Although the differences between milkings and the interaction between cows and milkings were not significant for solids-not-fat percentage, the differences among cows need further investigation. These differences among cows may be due to differences in milk yield, or any of the solid components. Hereditary influences should be considered, also.

## SUMMARY AND CONCLUSIONS

This study was conducted for the purpose of determining variations of the solid-milk components, between morning and evening milk of individual cows.

Forty purebred Holstein-Friesian cows were sampled monthly, during two consecutive milkings, over a period of three years. Milk samples were analyzed for fat, solids-not-fat, and total solids.

Significant differences among cows were obtained for pounds of milk, fat, solids-not-fat, and total solids, and percentage of fat, solids-not-fat, and total solids. A significantly larger quantity of milk was produced at the morning milking. No significant difference in percentage of solids-not-fat between morning and evening milk was found. Significant differences between morning and evening milk were obtained for pounds of fat, solids-not-fat, and total solids, and percentage of fat and total solids.

No evidence of interaction between cows and milking time was found in any part of this study.

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REFERENCES

(1) Bailey, G. L. Variations in the Solids-not-fat Fractions of Milk. *Dairy Sci. Abstr.*, **14**: 893. 1952.

(2) Bartlett, S. Variations in the Solids-not-fat Content of Milk. *J. Dairy Research*, **5**: 113. 1934.

(3) Eckles, C. H., and Shaw, R. H. The Influence of Breed and Individuality on the Composition and Properties of Milk. USDA, Bur. Animal Industry. *Bull.* 157. 1913.

(4) Herreid, E. O. Testing Milk and Milk Products for Fat and Total Solids. Univ. of Illinois, *Circ.* **630**: 61. 1948.

(5) Peterson, W. E. *Dairy Science*. 2nd ed. p. 340. J. B. Lippincott Co., Chicago, Illinois. 1950.

(6) Snedecor, George W. *Statistical Methods*. 5th ed., Iowa State College Press, Ames. 1956.

(7) Sprecht, L. W., Brunner, J. R., Madden, D. E., and Ralston, N. P. The Influence of Several Factors on the Protein and Solids-not-fat Content in the Milk of Jersey Cows. *J. Dairy Sci.*, **39**: 1337. 1956.

(8) Turner, C. W. Factors Affecting the Composition of Milk. Missouri Agr. Expt. Sta., *Bull.* 365. 1936.

(9) Wing, H. H. *Milk and Its Products*. p. 28. Macmillan Publ. Co., New York. 1913.

# EFFECTS OF DIETARY NaF ON DAIRY COWS. II. EFFECTS ON MILK PRODUCTION[1]

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**The objective of this investigation was to determine the levels of fluorine fed as sodium fluoride that would reduce milk production. Under adequate nutritional and husbandry conditions levels of 50 p.p.m. of fluorine caused no deleterious effect on the ability to produce milk in normal amounts. Average intakes of 1.5 and peak intakes of 1.7 mg. or more of F/kg. body weight reduced milk and fat production in certain cows as the result of anorexia which was accompanied by excessive loss of weight and stiffness in the legs. The cows tolerated 30 p.p.m. of fluorine, 40 p.p.m. was marginal in this respect while 50 p.p.m. caused fluorine toxicosis. It required two to five years after the fluorine effects were observed on the incisor teeth before the development of other physiologic effects appeared.** *Editor.*

A chronic fluorine toxicosis in dairy cows has been reported by some workers to result in decreased milk production. Phillips *et al. (8)* and Reed and Huffman *(12)* have reported an adverse effect on milk production when raw rock phosphate which contained fluorine was fed over an extended period. When lower levels of rock phosphate contaminated with fluorine were fed, Mather *(5)* and Elmslie *(4)* reported no effect on production. Recently, Dale and Crampton *(2)* have observed this decline in milk production in commercial dairy herds in Canada.

Rand and Schmidt *(11)* and Neeley and Harbaugh *(7)* observed no decrease in production in a field study of cattle ingesting natural water-borne fluorides.

More recently, contamination of forages by industrial effluents has focused attention on the effects of ingestion of a more soluble fluoride than that found in rock phosphate (Phillips *et al. (10)*]. Roholm *(13)* has reviewed many of the early cases of toxicity from this source. Blakemore and coworkers *(1)* reported that the milk production of cattle around English brick kilns was impaired, owing to the ingestion of fluorine-contaminated forage.

Although Dutoit *(3)* demonstrated decreased milk production when high levels of NaF (5 g. per day) were fed, there is a real need for information on the effect of low levels of a soluble fluoride on the long-term production of dairy cattle. Schmidt *et al. (14)*, in an interim report of their study on the feeding of NaF to dairy cattle on pasture, noted no effect on milk production.

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The principal objective of these studies was to determine the levels of fluorine fed as the soluble sodium salt which would reduce milk production.

## EXPERIMENTAL PROCEDURE

Bred two-year-old Holstein heifers were assigned to six lots as follows: I—Basal dairy ration; II—Basal + 20 p.p.m. fluorine as NaF; III—Basal + 30 p.p.m. fluorine as NaF; IV—Basal + 40 p.p.m. fluorine as NaF; V—Basal + 50 p.p.m. fluorine as NaF; VI—Basal + 50 p.p.m. fluorine as NaF + 200 gm. $CaCO_3$ per day.

The basis of this allotment, and the general husbandry practices employed, have been described *(9,15)*. The fluorine was administered as a solution poured over the grain ration once daily for the first year, and twice daily thereafter.

The pounds of milk produced were recorded daily and totaled monthly. Butterfat was determined once a month by the Babcock method and the pounds of fat produced were calculated. A record of the number of days in production was maintained for each cow.

Fluorine supplementation was begun February 7, 1951, and continued until the fall of 1956, when the sixth lactation was beginning. From rib biopsy data *(15)*, it appeared that there was a fluctuation in fluorine content of the skeleton, which was correlated with the stage of lactation. Because of this, an attempt was made to keep the number of cows slaughtered at different stages of lactation equally divided among lots. Some cattle were slaughtered on each of three different dates over a 4-mo. period. The average number of days on experiment was 2,050.

The effects of feeding fluorine, in the form of NaF, upon the milk production of dairy cows are shown by the data (compiled in Table 1). Records for the first lactation are partial, as the cows calved from December, 1950, to April, 1951, but fluorine supplementation for all cattle was begun on February 7, 1951.

## RESULTS

Inspection of the data (Table 1) reveals little if any adverse effects upon the milk production as the result of feeding NaF to young dairy cows. Further, the data indicate that following a latent period of two or more years, and depending upon the level of fluorine fed, milk production of certain cows was severely curtailed. Concurrent with the anorexia, weight loss, and lameness previously reported *(15)*, Cow 19 (Lot V, 50 p.p.m.), early in her third lactation, experienced a sharp reduction in milk production and upon contracting acute mastitis entirely dropped out of production. Cow 17 (Lot V, 50 p.p.m.) experienced similar difficulties in her fourth lactation. It is significant that these cows recovered after a prolonged rest period, and retained their ability to lactate at a normal rate. However, at the beginning of her sixth lactation, Cow 17's milk production dropped from 70 to 10 lb. in one day. She was subsequently placed in an outdoor lot on a blue grass turf for 2 wk., where she slowly regained production, appetite, and normal gait.

TABLE 1

*Summary of individual records by lactations*

| Lot and level F | COW | First lactation 1950-51 | | | Second lactation 1951-52 | | | Third lactation 1952-53 | | | Fourth lactation 1953-54 | | | Fifth lactation 1954-55 | | | Sixth lactation 1955-56 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MILK | FAT | DAYS | MILK | FAT | DAYS | MILK | FAT | DAYS | MILK | FAT | DAYS | MILK | FAT | DAYS | MILK | FAT | DAYS |
| | | *(lb.)* | | | | | | | | | | | | | | | | | |
| Lot I | 1 | 5,477 | 201 | 365 | — | — | —[a] | — | — | — | — | — | — | — | — | — | — | — | — |
| Control | 2 | 4,474 | 169 | 289 | 6,634 | 247 | 286 | 6,920 | 270 | 287 | 7,306 | 304 | 230 | 7,435 | 278 | 289 | 4,895 | 169 | 117[e] |
| | 3 | 6,310 | 237 | 306 | 7,159 | 266 | 264 | 8,503 | 311 | 282 | 12,584 | 498 | 368 | 12,403 | 455 | 373[d] | — | — | — |
| | 4 | 6,312 | 227 | 260 | 8,107 | 290 | 231 | — | — | —[b] | 9,898 | 367 | 288 | 10,448 | 408 | 366 | 4,992 | 199 | 97[e] |
| | 5 | 5,096 | 191 | 267 | 6,728 | 253 | 301 | 7,741 | 276 | 274 | 9,328 | 339 | 307 | 8,992 | 308 | 439 | 2,551 | 76 | 120[e] |
| Lot II | 6 | 5,058 | 167 | 267 | 4,867 | 160 | 254 | 6,690 | 244 | 274 | 9,862 | 372 | 309 | 7,819 | 299 | 293 | 5,569 | 202 | 141[e] |
| 20 p.p.m. | 7 | 7,269 | 237 | 283 | 8,347 | 237 | 284 | 10,120 | 333 | 321 | 9,832 | 305 | 181 | 7,363 | 235 | 310 | — | — | 25[f] |
| | 8 | 6,798 | 238 | 257 | 7,970 | 275 | 382 | 8,031 | 307 | 270 | 10,634 | 408 | 274 | 9,964 | 327 | 291 | 4,622 | 165 | 124[e] |
| | 9 | 5,919 | 209 | 237 | 9,160 | 301 | 359 | 13,631 | 499 | 420 | 1,725 | 84 | 34[a] | — | — | — | — | — | — |
| Lot III | 10 | 5,578 | 209 | 245 | 7,349 | 251 | 318 | 8,403 | 347 | 355 | 14,617 | 563 | 380 | 13,272 | 525 | 308 | — | — | —[g] |
| 30 p.p.m. | 11 | 6,431 | 203 | 245 | 9,167 | 269 | 322 | 11,311 | 377 | 313 | 11,475 | 412 | 306 | 6,200 | 167 | 146[c] | 10,831 | 333 | 225[e] |
| | 12 | 5,938 | 161 | 245 | 5,758 | 173 | 239 | 8,900 | 284 | 340 | 8,353 | 255 | 340 | 7,512 | 218 | 271 | 6,749 | 203 | 142[e] |
| | 13 | 6,167 | 183 | 287 | 10,815 | 307 | 366 | 10,134 | 350 | 280 | 12,090 | 359 | 300 | 16,442 | 568 | 421 | 2,672 | 107 | 45[e] |
| Lot IV | 14 | 9,846 | 326 | 485 | 10,100 | 363 | 396 | — | — | —[b] | 12,627 | 410 | 396 | 12,870 | 425 | 345 | 2,066 | 68 | 27[e] |
| 40 p.p.m. | 15 | 5,691 | 243 | 299 | 6,690 | 288 | 331 | 7,010 | 297 | 254 | 7,737 | 369 | 275 | 5,061 | 247 | 102[c] | 689 | 33 | 20[a] |
| | 16 | 3,539 | 113 | 218 | 4,490 | 143 | 199 | 3,967 | 149 | 235 | 8,312 | 278 | 288 | 7,737 | 248 | 318 | 8,679 | 280 | 179[e] |
| | 17 | 7,014 | 190 | 245 | 8,805 | 258 | 293 | 8,066 | 243 | 335 | 3,438 | 101 | 69[c] | 14,279 | 488 | 388 | 5,486 | 184 | 123[e] |
| Lot V | 18 | 5,501 | 188 | 245 | — | — | —[a] | — | — | — | — | — | — | — | — | — | — | — | — |
| 50 p.p.m. | 19 | 9,864 | 299 | 384 | 12,690 | 370 | 372 | 1,514 | 60 | 35[c] | — | — | —[b] | 23,998 | 735 | 581[d] | — | — | — |
| | 20 | 9,416 | 295 | 306 | 7,297 | 282 | 257 | 8,905 | 358 | 313 | 13,022 | 530 | 307 | 15,187 | 555 | 411 | 3,606 | 148 | 80[e] |
| | 21 | 5,932 | 195 | 245 | 9,166 | 305 | 342 | 7,443 | 276 | 314 | — | — | —[a] | — | — | — | — | — | — |
| Lot VI | 22 | 6,795 | 219 | 245 | 8,187 | 261 | 284 | 8,489 | 283 | 300 | 11,067 | 342 | 280 | 10,289 | 333 | 280 | 11,652 | 371 | 219[e] |
| 50 p.p.m. | 23 | 7,775 | 281 | 336 | 6,361 | 236 | 287 | 8,704 | 304 | 269 | 9,967 | 374 | 276 | 11,860 | 393 | 341 | 9,159 | 363 | 181[e] |
| + $CaCO_3$ | 24 | 7,252 | 293 | 267 | 7,038 | 246 | 297 | 8,768 | 333 | 270 | 10,271 | 399 | 302 | 12,335 | 483 | 387 | 4,306 | 183 | 92[e] |

[a] Died, see text.

[b] Breeding difficulties, missed this lactation.

[c] Acute mastitis early in lactation, out of production.

[d] Breeding difficulties, slaughtered near end of fifth lactation for termination data.

[e] Slaughtered for termination data during sixth lactation.

[f] Developed mastitis after freshening, no milk saved, slaughtered for termination data.

[g] No sixth lactation, slaughtered while dry for termination data.

Cows 13, 16, 22, and 24 exhibited some physiological effects of fluorine during the fifth and sixth lactations *(15)*. However, their milk production during these periods was not depressed.

This suggests that 40 to 50 p.p.m. fluorine, fed as NaF for 5½ yr., was sufficiently toxic to produce some fluorotic symptoms, but it did not consistently produce an effect on the milk-producing capacity of these well-fed dairy cows.

The data presented (Table 1) have been summarized to present the total milk, butterfat, and days milked per cow during the experimental period (Table 2). It is apparent that the feeding of as much 50 p.p.m. fluorine as NaF did not adversely affect the total pounds of milk or butterfat produced during the 5½-yr. period; nor was the total number of days in lactation per cow markedly

TABLE 2

*Summary of effect of fluorine fed as NaF on the total milk, fat, and days milked per cow for five and one-half years*

| Lot and treatment | Av. milk[a] | Av. fat[a] | Av. days[a] |
|---|---|---|---|
| I — Control | 41,460 | 1,565 | 1,444 |
| II — 20 p.p.m. F | 42,813 | 1,489 | 1,562 |
| III — 30 p.p.m. F | 49,261 | 1,650 | 1,580 |
| IV — 40 p.p.m. F | 48,858 | 1,539 | 1,517 |
| V — 50 p.p.m. F | 50,862 | 1,699 | 1,499 |
| VI — 50 p.p.m. F + $CaCO_3$ | 53,425 | 1,899 | 1,638 |

[a] Does not include data on Cows 1, 9, 18, and 21, which died during the experiment.

different between the lots fed the various levels of fluorine. These data do not fully support an earlier progress report by Miller *(6)*; namely, that 50 p.p.m. of fluorine fed as NaF would reduce both days in lactation and total milk produced. The excellent recovery, and subsequent return to lactation, of Cows 17 and 19 were responsible for the differences noted between this and the previous report.

The absence of any effect on milk production in the third cow in Lot V (No. 20) can be explained by the lower intake, on an mg. F/kg body weight basis, as compared to the other two cows which received 50 p.p.m. fluorine.

Because of the relationship and importance of both the time element and the ingestion level of fluorine in the development of fluorosis, summary data on the mg. F ingested/kg body weight are presented (Table 3).

Comparison of these data with the symptomatology described above, shows that when dairy cattle were subjected to sustained ingestion levels of 1.5, and peak levels of from 1.7 to 1.8 mg. F/kg of body weight, a fluorine toxicosis resulted in certain cows, which was characterized by sharp and prolonged lapses in milk production. These cattle were first exposed as two-year-olds, and continuously thereafter for over 5 yr. Levels of fluorine somewhat lower than this resulted in some evidence of toxicity, but were without effect on milk production *(15)*.

The onset of the critical period in all cases followed a few weeks at the peak ingestion levels indicated above. Anorexia specifically directed toward NaF-con-

taining feeds reduced the feed consumed, and consequently the fluorine intake was reduced to tolerated, and less toxic, levels. Likewise, the latter half of the lactation period and the dry period permitted some degree of recovery from the peak ingestion periods and would, therefore, extend the time-interval necessary to produce fluorosis.

These data suggest that under good nutritional and husbandry practices fluorine levels as high as 50 p.p.m., fed in the form of NaF, caused no direct interference with milk secretion. They also suggest that a critical or upper limit

TABLE 3

*Fluorine ingestion (mg. F/kg of body weight)*

| Lot | Cow No. | Added F intake — by year (mg/kg body weight) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 Peak | Av. | 2 Peak | Av. | 3 Peak | Av. | 4 Peak | Av. | 5 Peak | Av. |
| I Control | | | | | | | | | | | |
| II | | | | | | | | | | | |
| 20 p.p.m. F | 5 | 0.64 | 0.54 | 0.65 | 0.51 | 0.67 | 0.55 | 0.64 | 0.53 | 0.64 | 0.48 |
| | 6 | 0.69 | 0.56 | 0.64 | 0.52 | 0.60 | 0.50 | 0.72 | 0.52 | 0.59 | 0.50 |
| | 7 | 0.77 | 0.56 | 0.63 | 0.55 | 0.66 | 0.54 | 0.70 | 0.59 | 0.47 | 0.40 |
| | 8 | 0.82 | 0.59 | 0.72 | 0.61 | 0.65 | 0.53 | 0.64 | 0.55 | 0.61 | 0.52 |
| III | | | | | | | | | | | |
| 30 p.p.m. F | 9 | 0.97 | 0.86 | 1.08 | 0.89 | 1.03 | 0.90 | 1.03 | 0.72 | — | — |
| | 10 | 0.95 | 0.81 | 1.05 | 0.82 | 0.92 | 0.81 | 0.91 | 0.76 | 0.85 | 0.63 |
| | 11 | 1.03 | 0.88 | 1.14 | 0.91 | 1.06 | 0.91 | 1.13 | 0.89 | 1.05 | 0.81 |
| | 12 | 0.99 | 0.84 | 1.11 | 0.86 | 0.95 | 0.80 | 0.92 | 0.77 | 0.89 | 0.77 |
| IV | | | | | | | | | | | |
| 40 p.p.m. F | 13 | 1.37 | 1.21 | 1.59 | 1.26 | 1.42 | 1.13 | 1.41 | 1.15 | 1.32 | 1.13 |
| | 14 | 1.20 | 1.11 | 1.19 | 1.10 | 1.09 | 0.96 | 1.14 | 0.99 | 1.10 | 0.88 |
| | 15 | 1.32 | 1.09 | 1.32 | 1.13 | 1.27 | 1.09 | 1.26 | 1.02 | 1.26 | 0.98 |
| | 16 | 1.24 | 1.00 | 1.19 | 1.10 | 1.10 | 0.94 | 1.19 | 1.00 | 1.30 | 0.96 |
| V | | | | | | | | | | | |
| 50 p.p.m. F | 17 | 1.64 | 1.47 | 1.85 | 1.60 | 1.90 | 1.44 | 1.82 | 1.56 | 1.79 | 1.53 |
| | 19 | 1.70 | 1.51 | 1.81 | 1.57 | 1.76 | 1.44 | 1.47 | 1.22 | 1.91 | 1.54 |
| | 20 | 1.35 | 1.22 | 1.48 | 1.20 | 1.45 | 1.20 | 1.62 | 1.29 | 1.56 | 1.29 |
| VI | | | | | | | | | | | |
| 50 p.p.m. F | 21 | 1.78 | 1.44 | 1.75 | 1.47 | 1.53 | 1.37 | — | — | — | — |
| + $CaCO_3$ | 22 | 1.47 | 1.27 | 1.71 | 1.44 | 1.52 | 1.40 | 1.69 | 1.41 | 1.58 | 1.31 |
| | 23 | 1.69 | 1.50 | 1.70 | 1.64 | 1.66 | 1.42 | 1.63 | 1.33 | 1.56 | 1.29 |
| | 24 | 1.71 | 1.30 | 1.68 | 1.44 | 1.64 | 1.44 | 1.70 | 1.29 | 1.53 | 1.35 |

of fluorine intake was reached at approximately 1.7 mg. F per kg. of body weight. The stress of early lactation, with its inherent elevation of feed intake, caused certain cows to reach this level of fluorine ingestion, which, if long sustained, resulted in an effect on appetite and excessive weight loss. With curtailed feed consumption, lactating animals were forced eventually to a lowered level of milk production. These results suggest that the effect of fluorine fed as NaF on milk production is a secondary one, closely correlated with curtailed feed intake, or starvation phenomena.

The addition of calcium carbonate to the ration of the cows in Lot VI seemed to reduce the toxicity of 50 p.p.m. of added fluorine about 10 p.p.m. These cows in Lot VI were consistently regular in reproduction and milk production, with no indication of any adverse effect on milk or butterfat production.

SUMMARY

A study of the tolerance of lactating cows to added dietary increments of a soluble fluoride (NaF) over a 5½-yr. period has been made. The results indicate that under adequate nutritional and husbandry conditions, levels of 50 p.p.m. of fluorine fed as NaF produced no direct deleterious effect upon milk secretion. Sustained fluorine ingestion, with average intake levels of 1.5, and peak intakes of 1.7 or more, mg. of F/kg body weight, led to a reduction of milk and butterfat production in certain cows, as the result of anorexia toward NaF-containing feed, which was accompanied by excessive weight loss and stiffness in the legs. These results indicate further that the effect of fluorine fed in the form of NaF on milk production was a secondary effect conditioned by a curtailed feed intake. The cows in this study tolerated 30 p.p.m. fluorine in their ration, whereas 40 p.p.m. was apparently near the marginal zone of tolerance and 50 p.p.m. in the ration resulted in the development of fluorine toxicosis. The addition of calcium reduced the toxicity of the added NaF.

A latent period of from 2 to 5 yr. elapsed between the time the fluorine effects were first noticed on the incisor teeth and the development of other physiologic effects, including the inhibition of milk production. Thus, these studies contribute further evidence to show that the development of fluorine toxicosis is a function of the duration of exposure (time), as well as of level of ingestion. Prolonged dry periods allowed sufficient recovery from debilitating fluorosis for subsequent normal lactation and milk production.

REFERENCES

(1) Blakemore, F., Bosworth, T. J., and Green, H. H. Industrial Fluorosis of Farm Animals in England, Attributable to the Manufacture of Bricks, the Calcining of Ironstone, and to Enameling Processes. *J. Comp. Path.*, **58**: 267. 1948.

(2) Dale, D. G., and Crampton, E. W. Observations on Chronic Fluorosis in Dairy Cattle. *Can. J. Comp. Med.*, **19**: 6. 1955.

(3) Dutoit, P. J., Malan, A. I., Groenewald, J. W., and DeKock, G. W. Studies in Mineral Metabolism. XXVI. The Effects of Fluorine on Pregnant Heifers. 18th Rept., *Dir. of Vet. Services and Animal Indus. Union of S. Africa.* 1932.

(4) Elmslie, W. P. Effect of Rock Phosphate on Dairy Cows. *Proc. Am. Soc. Animal Prod.*, pp. 44-48 (29th Annual Meeting). 1936.

(5) Mather, R. E., Pratt, A. D., and Holdaway, C. W. Fused Tricalcium Phosphate as a Low-Fluorine Phosphorous Supplement for Dairy Cattle. *J. Dairy Sci.*, **32**: 228. 1949.

(6) Miller, R. F. Studies on the Mode of Action and Development of Fluorosis in Cattle and Laboratory Animals. Ph.D. thesis, University of Wisconsin. 1955.

(7) Neeley, K. L., and Harbaugh, F. G. Effects of Fluoride Ingestion on a Herd of Dairy Cattle in the Lubbock, Texas Area. *J. Am. Vet. Med. Assoc.*, **124**: 344. 1954.

(8) Phillips, P. H., Hart, E. B., and Bohstedt, G. Chronic Toxicosis in Dairy Cows Due to the Ingestion of Fluorine. Wis. Agr. Expt. Sta., *Research Bull.* 123. 1934.

(9) Phillips, P. H. The Development of Chronic Fluorine Toxicosis and Its Effect on Cattle. *Proc. 2nd Natl. Air Pollution Symposium.* Pasadena, California. 1952.

(10) Phillips, P. H., Greenwood, D. A., Hobbs, C. S., and Huffman, C. F. The Fluorosis Problem in Livestock Production. *National Research Council Publication* 381. 1955.

(11) Rand, W. E., and Schmidt, H. J. The Effect upon Cattle of Arizona Waters of High Fluoride Content. *Am. J. Vet. Research*, **8**: 50. 1952.

(12) Reed, O. E., and Huffman, C. F. The Results of a Five-Year Mineral Feeding Investigation with Dairy Cattle, Mich. State Coll. Agr. Expt. Sta., *Tech. Bull.* 105. 1930.

(13) Roholm, K. *Fluorine Intoxication, a Clinical-Hygienic Study, with a Review of Literature and Some Experimental Investigations.* H. K. Lewis and Co., London, England. 1937.

(14) Schmidt, H. J., Newell, G. W., and Rand, W. E. The Controlled Feeding of Fluorine, as Sodium Fluoride, to Dairy Cattle. *Am. J. Vet. Research*, **15**: 232. 1954.

(15) Suttie, J. W., Miller, R. F., and Phillips, P. H. Studies of the Effects of Dietary NaF on Dairy Cows. I. The Physiological Effects and the Development Symptoms of Fluorosis. *J. Nutrition*, **63**: 211. 1957.

# TECHNICAL NOTES

## SINGLE VERSUS DUPLICATE DETERMINATIONS FOR THE ESTIMATION OF VITAMIN A AND TOCOPHEROL IN CALF LIVERS[1]

In calf nutrition studies, in which the objective is to determine the effect of different treatments, e.g., on the concentration of vitamin A or tocopherol of the liver, the error variability used in statistical tests of significance consists of two general sources: differences in the liver concentrations between calves on the same treatment, calf variability, and differences within the liver of a single calf, such as those due to between-samples from the same liver, between-duplicate determinations from a single sample, etc., measurement variability. If the first source of variability is relatively large in comparison with the second, little reduction in the error variability is accomplished by increasing the number of samples for a particular liver, or the number of determinations per sample. Data from eight randomized-block experiments, conducted during 1952-56, in which calves were fed fixed levels of vitamin A and/or its precursor, carotene, and/or tocopherol for periods ranging from 4 to 16 wk., were used to ascertain the effect on the error variability of using the first duplicate determination for vitamin A or tocopherol concentration, as contrasted to using the average of the first and second duplicates.

The liver from each calf, obtained at the termination of each calf's experimental period, was trimmed of large arteries, veins, and external fat, and macerated in a Hobart food-chopper. Representative samples were taken and held at 0° F. for subsequent analyses. Vitamin A was determined in duplicate from a single sample by the Davies Procedure and/or by a modification of the Gallup-Hoefer procedure *(5)*. Total tocopherols were also determined by a modification *(4)* of the method of Quaife and Harris *(8)*, in which the molecular distillates were chromatographed on Floridin (Florex, XXS, 60-90 mesh, Floridin Co., Tallahassee, Fla.) to remove interfering compounds *(3)*, rather than employing selective hydrogenation *(8)*. The duplicate samples, differing in weight, were run side by side. Because of differing sample weights, bias in the magnitude of the difference between duplicate samples was reduced to some extent, for the analyst recorded galvanometer deflections for an entire day's run prior to calculation of the concentrations.

The data reported herein, which were statistically analyzed according to procedures as outlined by Cochran and Cix *(7)* and by Snedecor *(11)*, represent unselected duplicate determinations from a single representative liver sample, with the exception of determinations which had to be repeated due to analytical mistakes. Values of the individual determinations for vitamin A and tocopherol were each transformed to their respective logarithms (Base 10) to eliminate the increase in the error variability (calf plus measurement) of these criteria, associated with increasing intake of either vitamin by the calf as previously discussed *(9, 10)*, as well as a similar increase in the measurement variability (difference between duplicates) associated with increasing concentration of either vitamin in the liver sample. The logarithmic transformation was chosen because the variance of liver vitamin A or tocopherol concentration by intake groups was essentially proportional to the mean concentration squared; also, the variance of the duplicates was essentially proportional to the mean liver vitamin A or tocopherol concentration of the sample squared.

The error mean squares (treatments × blocks) derived from separate analyses of variance for each experiment, using in the first case—column four—the value from the first duplicate, $M.S._1$ and, in the second case—column five—the average value of the duplicates, $M.S._2$, are given (Table 1). In general, the mean squares of the average of the duplicate values were slightly lower in magnitude than those in which only the first duplicate value was used. The ratios of $M.S._1$ to $M.S._2$, column six, were, with the exception of Experiment IV for liver vitamin A and Experiment VII for liver tocopherol, greater than 1.00, indicating also some reduction in the magnitude of the error. However, the *t*-values of these ratios, column seven, using the Pitman-Morgan test for correlated mean squares *(6)*, indicated that the reductions in the size of the experimental errors, due to using the average of the duplicates, were not statistically significant. One exception was in the case of Experiment IV, in which use of the average of the duplicates increased the size of the error significantly. The direct computation of error mean squares, using the first duplicate, and the direct computation of error mean squares using the average duplicates, and comparing these by the Pitman-Morgan test, were

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TABLE 1

*Effect of using the first duplicate determination and the average of duplicate determinations on the error mean squares of vitamin A and tocopherol concentrations of calf liver*

| Experiment | No. of calves | Range of values | Error mean square: Using value of first duplicate only ($M.S._1$) | Error mean square: Using av. of two duplicates ($M.S._2$) | Ratio of mean squares | $t$-value[a] | Mean for the expt.[b] | Standard deviation of a single determination |
|---|---|---|---|---|---|---|---|---|
| Liver vitamin A | | *(γ/100 g.)* | *(log[γ/100 g.])* | | *($M.S._1/M.S._2$)* | | *log [(γ/100 g.)]* | |
| Davies procedure | | | | | | | | |
| I | 40 | 6 to 622 | 0.0356 | 0.0347 | 1.02 | 0.683 (25)[c] | 1.74 | 0.022(39)[c] |
| II | 24 | 57 to 2,821 | 0.0333 | 0.0304 | 1.10 | 2.063 (14) | 2.72 | 0.022(24) |
| III | 36 | 14 to 4,703 | 0.0602 | 0.0592 | 1.02 | 0.462 (18) | 2.61 | 0.022(33) |
| IV | 48 | 12 to 8,138 | 0.0946 | 0.0981 | 0.96 | 2.736*(27) | 2.59 | 0.020(46) |
| Modified Gallup-Hoefer procedure | | | | | | | | |
| IV | 48 | 7 to 7,817 | 0.1006 | 0.1037 | 0.97 | 2.964*(27) | 2.58 | 0.013(46) |
| V | 9 | 321 to 77,111 | 0.0248 | 0.0241 | 1.03 | 0.153 (2) | 3.74 | 0.016(9) |
| VI | 18 | 124 to 91,400 | 0.0301 | 0.0294 | 1.02 | 0.841 (7) | 3.66 | 0.009(18) |
| Liver tocopherol | | *(mg/100 g.)* | *(log[(mg/100 g.)(100)])* | | | | *(log[(mg/100 g.)(100)])* | |
| III | 36 | 0.155 to 0.549 | 0.0123 | 0.0109 | 1.13 | 0.615 (20) | 1.45 | 0.067(35) |
| V | 9 | 0.405 to 11.356 | 0.0218 | 0.0185 | 1.18 | 0.600 (2) | 2.24 | 0.047(9) |
| VI | 18 | 0.441 to 14.539 | 0.0096 | 0.0056 | 1.71 | 1.480 (7) | 2.30 | 0.069(18) |
| VII | 8 | 0.187 to 5.532 | 0.0110 | 0.0147 | 0.74 | 0.713 (2) | 2.08 | 0.057(8) |
| VIII | 40 | 0.056 to 0.840 | 0.0114 | 0.0107 | 1.06 | 0.360 (26) | 1.30 | 0.072(40) |

*Indicates significance at $P \leqslant 0.05$.
[a] Pitman-Morgan test for correlated mean squares applied, Cochran *(6)*.
[b] Based on average of duplicate determinations.
[c] Error degrees of freedom.

undertaken in contrast to the components-of-variance approach, because duplicates were run side by side in contrast to random order.

Whether the individual calf-liver concentration values differed when using the first duplicate, as compared to using the average of the duplicates, was explored by derivation of linear regressions of the average of the duplicates, minus the first duplicate on the average of the duplicates. Separate regressions for each experiment and across all experiments by procedures for vitamin A and for tocopherol indicated only random variability, i.e., neither the intercept nor the slope of these regressions differed significantly from zero. Over-all means for each experiment, based on the average of the duplicates, are given (column 8—Table 1). Means based on the first duplicate differed from means based on the average of the duplicates by not more than 0.01; therefore, the values of the former are not given.

These statistics would appear to indicate that a single determination of liver vitamin A or tocopherol concentration is sufficient in calf nutrition studies, in which the determination of treatment differences is the main consideration. These statistics, however, might not apply to experiments in which monozygotic twins are being used. For example, Boyne and Greig *(2)* have recently shown, using hematological data, that variability associated with twin calves is considerably less of the total variability than is the case when calves other than monozygotic twins are used. Thus, considerably greater reduction in the error variability, as a result of using the average of duplicates, in contrast to using a single determination, would be expected in monozygotic twins than in nonmonozygotic calves. Another consideration is the laboratory control over the measurement portion of the error. Single determinations would not allow control over the measurement portion of the error. In the interest of laboratory control, it would be good practice, as in the determination of liver tocopherol concentration which involves several steps, to include one or more sets of duplicates (preferably at random) and compare the difference between the duplicates with a previously established control limit, usually three times the estimate of the standard deviation of a single determination, times the square root of two, $3s\sqrt{2}$, *(1)*.

Estimates of the standard deviation of a single determination were derived for control purposes (column 9, Table 1), by analysis-of-variance (variation within livers from the same calf) procedures. With the exception of the modified Gallup-Hoefer method for vitamin A,

the variances thus obtained were homogenous, as indicated by Bartlett's test of homogeneity of variance *(11)*. In the case of the Gallup-Hoefer procedure, the variances were suspected of not being homogenous, $P < 0.10 > 0.05$. Average standard deviations of a single determination, weighted on the basis of degrees of freedom used in the estimate (numbers in parentheses, column 9, Table 1), were for the Davies procedure for the estimation of liver vitamin A, 0.021 in log ($\gamma$/100 g.), for the modified Gallup-Hoefer procedure for vitamin A, 0.012 in log ($\gamma$/100 g.), and for tocopherol, 0.067 in log [(mg/100 g.)(100)]. Multiplying these average estimates of s by $3\sqrt{2}$, the $\sqrt{2}$ being used since the estimate is based on two observations *(1)*, the analyst would reject a run, or sample, in which the difference between duplicates was greater than 0.089 log ($\gamma$/100 g.) for the Davies vitamin A procedure, 0.051 log ($\gamma$/100 g.) for the modified Gallup-Hoefer procedure for vitamin A, and 0.284 log [(mg/100 g.)(100)] for tocopherol.

A graphic method of control has been proposed by Bennett and Franklin *(1)* (Figure 10.9 of their text). By using 2-phase, 3-cycle log graph paper, control charts can be constructed so as to eliminate the necessity of the logarithmic transformation. An example of this is given (Figure 1 of this paper) for the

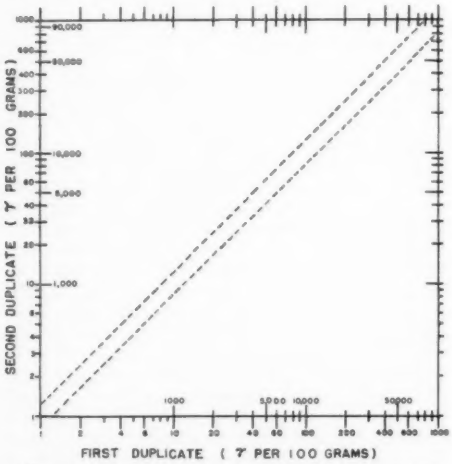


FIG. 1. Control chart for accepting or rejecting duplicate analyses, in the estimation of liver vitamin A by the Davies procedure (see text of paper for description of its use).

Davies procedure for liver vitamin A. When the concentration of the first duplicate is plotted against the concentration of the second duplicate, the point of intersection of the two perpendicular lines representing these plots, to be acceptable, must fall within the broken (- - -) lines. Rejection of duplicates indicates laboratory variation of an unexpected magnitude. Careful evaluation of analytical procedures for that particular run, as well as possibly running all samples in duplicate for the next several runs, would be advisable. Once the cause of variation is removed, returning to the previous procedure, of including only one set of duplicates in each run, would seem feasible.

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## REFERENCES

(1) BENNETT, C. A., AND FRANKLIN, N. L. *Statistical Analysis in Chemistry and the Chemical Industry.* Chap. 10. John Wiley and Sons, Inc., New York. 1954.

(2) BOYNE, A. W., AND GREIG, W. A. The Value of Repeated Observations in Biological Sampling. *J. Agr. Sci.*, **47**: 145. 1956.

(3) BROWN, F. The Estimation of Vitamin E. I. Separation of Tocopherol Mixtures Occurring in Natural Products by Paper Chromatography. *Biochem. J.*, **51**: 237. 1952.

(4) BUNNELL, R. H. The Effect of Fish Oil and Diphenyl-*p*-phenylenediamine on the Vitamin E Metabolism of the Chick. Ph.D. thesis. University of Connecticut, Storrs. 1955.

(5) BUNNELL, R. H., ROUSSEAU, J. E., JR., EATON, H. D., AND BEALL, G. Estimation of Vitamin A and Carotenoids in Calf Liver. *J. Dairy Sci.*, **37**: 1473. 1954.

(6) COCHRAN, W. G. Query. *Biometrics*, **1**: 71. 1945.

(7) COCHRAN, W. G., AND COX, GERTRUDE M. *Experimental Designs.* John Wiley and Sons, Inc., New York. 1950.

(8) QUAIFE, MARY L., AND HARRIS, P. L. Chemical Assay of Foods for Vitamin E Content. *Anal. Chem.*, **20**: 1221. 1948.

(9) ROUSSEAU, J. E., JR., DICKS, MARTHA W., TEICHMAN, R., HELMBOLDT, C. F., BACON, E. L., PROUTY, R. M., DOLGE, K. L., EATON, H. D., JUNGHERR, E. L., AND BEALL, G. Relationship Between Plasma, Liver and Dietary Tocopherol in Calves, Lambs and Pigs. *J. Animal Sci.*, **16**: 612. 1957.

(10) ROUSSEAU, J. E., JR., EATON, H. D., TEICHMAN, R., HELMBOLDT, C. F., JUNGHERR, E. L., BACON, E. L., AND BEALL, G. Relative Value of Carotene from Alfalfa and Vitamin A from a Dry Carrier Fed at Medium to High Levels to Holstein Calves. *J. Dairy Sci.*, **39**: 1565. 1956.

(11) SNEDECOR, G. W. *Statistical Methods.* 5th ed. Iowa State College Press, Ames. 1956.

## COLORIMETRIC DETERMINATION OF CITRATE IN MILK BY BABAD AND SHTRIKMAN

One of the common methods employed for the determination of citrate in milk involves its oxidation by potassium permanganate to acetone dicarboxylic acid which, in turn, in the presence of bromine, is converted to pentabromacetone and estimated gravimetrically *(2)*. However, Babad and Shtrikman *(1)* have developed a colorimetric method, modified for the citrate determination in milk and other dairy products, based on the method employed for blood by Saffran and Denstedt *(4)*. They contend that this method is more satisfactory than the gravimetric method; it is more rapid and accurate, because the pentabromacetone is volatile when wet and often is obtained as an oil, rather than in a crystalline form. In their method, a final concentration of 5% (v/v) trichloracetic acid is used for the precipitation of proteins and the reaction mixture is allowed to stand for 2 hr. before filtration. An aliquot of 0.2 ml. of the filtrate containing citrate is heated with 7 ml. of dry acetic anhydride at 60° C. for 10 min.; then, 1 ml. of pyridine is added and the solution is heated for an additional period of 40 min. The permanent yellow color developed is estimated colorimetrically at 420 mμ.

In employing this procedure in our laboratory, we observed that whereas the gravimetric method gave consistent results, both with pure citric acid and milk, the colorimetric method gave high results for milk (Table 1). Rowland *(3)* has shown that, unlike in blood, a final concentration of 12% trichloracetic acid is necessary for complete precipitation of proteins from milk. Therefore, we increased the final concentration of trichloracetic acid in the colorimetric citrate analysis to 12%, and compared this modified procedure with the gravimetric method. These results indicate satisfactory agreement between the two methods and suggest that the high results obtained by the unmodified procedure of Babad and Shtrikman were due to incomplete precipitation of proteins. It was also observed that the use of rubber stoppers for the test tubes was unsatisfactory.

On the basis of these observations, the following modifications in their method are suggested: *(a)* a final concentration of 12% trichloracetic acid to precipitate the proteins before filtration, and *(b)* glass-stoppered test tubes for color development.

TABLE 1

*A typical comparison of the colorimetric and gravimetric methods for citrate analysis of milk*

| Milk Sample 1 | | Milk Sample 2 | |
|---|---|---|---|
| Gravimetric method | Colorimetric method | Gravimetric method | Colorimetric method (modified) |
| (meq/l) | | | |
| 24.07 | 27.04 | 27.77 | 28.11 |
| 24.45 | 27.12 | 27.92 | 28.08 |

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REFERENCES

(1) Babad, J., and Shtrikman, N. The Estimation of Citric Acid in Dairy Products. *J. Dairy Research*, **18**: 72. 1951.

(2) Lampitt, L. H., and Rooke, H. S. The Citric Acid in Milk and Its Determination. *Analyst*, **61**: 654. 1936.

(3) Rowland, S. J. The Determination of the Nitrogen Distribution in Milk. *J. Dairy Research*, **9**: 42. 1938.

(4) Sáffran, M., and Denstedt, O. F. A Rapid Method for the Determination of Citric Acid. *J. Biol. Chem.*, **175**: 849. 1948.

## MODIFICATIONS OF SAMPLING PROCEDURES FOR DAIRY HERD IMPROVEMENT ASSOCIATION SUPERVISORS [1]

The work of the D.H.I.A. supervisor, in compiling and processing data for the farmer, is laborious and tedious. The use of automatic computers relieves the supervisor of much routine bookkeeping.

The purpose of this Note is to report the results of attempts to reduce the time involved in conventional sampling procedures, by removing a proportionate sample of milk from the milk pail and placing it in the milk-test

[1] Authorized for publication on May 15, 1957, as paper No. 2150 in the Journal Series of the Agricultural Experiment Station.

bottle at each milking. Two comparative studies were made. In each study, the control tests were made by two D.H.I.A. supervisors, each of whom had more than 20 years of experience. In both studies, the samples taken by the modified procedures were delivered to the Dairy Science Department, where they were tested.

The first study, which included 853 comparative tests, consisted of removing from the thoroughly mixed milk in the pail, after the evening milking, 8.8 ml. of milk with an 8.8-ml. pipette and introducing it into a test bottle. The same amount was removed from the morning milk and added to the same test bottle used for the previous evening's sample. The second study, which included 621 comparisons, was similar to the first, but made use of the Cornwall Leur-Lok syringe with metal holder, plunger, spring-washer, and extension. This semi-automatic syringe is graduated to a total of 10 ml. in 0.5-ml. increments and was adjusted in the laboratory to deliver 8.8 ml. of milk. During the night and morning milkings, the milk was removed with the syringe and delivered into the test bottle. Approximately equal numbers of samples were submitted by each of the two D.H.I.A. supervisors involved in both studies.

A statistical analysis was made of each procedure, in comparison with the routine tests made on the farms. Statistical data for each sampling method is given below:

| | Method 1 *(8.8-ml. pipette)* | Method 2 *(8.8-ml. syringe)* |
|---|---|---|
| $N$ | 853 | 621 |
| $\overline{X}$ | 4.40 | 4.40 |
| $\overline{Y}$ | 4.39 | 4.45 |
| $r$ | 0.9925 | 0.9905 |
| $SY \cdot X$ | 0.092 | 0.109 |

Where

$N$ = number of comparisons

$\overline{X}$ = Average of Dairy Herd Improvement Association Supervisors' control tests

$\overline{Y}$ = Average tests of modified sampling procedures

$r$ = Coefficient of correlation

$SY \cdot X$ = Standard error of estimate of $Y$ based on $X$, including average discrepancy.

Thus, if the conventional test is 4.0%, the test obtained by experimental method No. 1 at the 95% confidence limit would be between 3.82 and 4.18%. In experimental method No. 2, a 4.0% test at the 95% confidence limit would be between 3.79 and 4.21. The average deviation for Method 1 was $\pm$ 0.079 and for Method 2 was $\pm$ 0.089. These average deviations agree with those expected from standard errors of estimate from these correlations.

The data revealed further that in the comparison of Method 1 with the normal procedure, 86.6% of the tests fell within 0.1% of each other; whereas, in Method 2, 81.3% of the results were in a similar range. In Method 1, 97.6% of the results checked within 0.2% and in Method 2, 96.3% of the results were in this category.

These sampling procedures produce results which are accurate enough for practical purposes, but a question may arise concerning their use on farms, where the interval between milking is varied enough to make a considerable difference in the quantity of milk produced and the fat test. Roadhouse and Henderson *(2)* published data covering ten days of milking at 12-hr. intervals, in which the minimum and maximum production per cow was 13.0 and 16.6 lb., respectively, or a maximum variation of 3.6 lb. during the period. During a 14- and 10-hr. interval milking experiment, the minimum and maximum production per cow was 11.3 and 19.3 lb., respectively, or a maximum variation of 8.0 lb. during the five-day experiment. They also showed the fat tests varied a maximum of 0.28% on equal intervals and 0.13% on 14- and 10-hr. intervals. Others *(1, 3)* have suggested variations in quantity and fat test where the interval between milking varies widely. Obviously, the use of an 8.8-ml. pipette or syringe precludes the use of aliquot sampling. Nearly all the herds in this study were being milked at approximately 12-hr. intervals.

It is felt that the sampling procedures studied herein might be employed conveniently by D.H.I.A. supervisors, and also by the farmer using the owner-sampler program for dairy herd improvement, providing the interval between milking is approximately 12 hr. Less milk would be used, the chance of losing samples due to souring or churning would be lessened, and the added work of mixing and pipetting them would also be decreased.

ACKNOWLEDGMENTS

The author wishes to express his appreciation to Mr. Thomas Fox and Mr. Ira Whiteman for their assistance in securing the data, and to Dr. Leonard Mattick for his advice and assistance in the statistical analysis.

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## REFERENCES

(1) HERRINGTON, B. L. *Milk and Milk Processing.* McGraw-Hill Book Company, N. Y. 1948.

(2) ROADHOUSE, C. L., AND HENDERSON, J. L. The Lactose and Chloride Concentration of Milk Produced During Irregular Intervals Between Milking. *J. Dairy Sci.*, **15**: 1. 1932.

(3) SOMMER, H. H. *Market Milk and Related Products.* Olson Publishing Company, Milwaukee, Wis. 1952.

# SYMPOSIUM

## LEGAL AND REGULATORY ASPECTS OF DAIRY PRODUCTS MANUFACTURE

This symposium was held at the 52nd Annual Meeting of the American Dairy Science Association at Oklahoma State University, Stillwater.

The breadth, depth, and international scope of the discussion of the legal and regulatory aspects of dairy products manufacture was a timely part of the program. Problems were discussed that affect nations concerned in developing foreign trade for either imports or exports of dairy products. The speakers are commended for their fruitful efforts and the program committee for its vision in planning this symposium.

Consideration should be given to sponsoring by the Association of more symposia on specialized subjects. They need not, necessarily, be held at the time of the annual meeting but at such places and times where it might be most convenient, timely, and pleasant. This is an activity of the Association that should be considered for further expansion. *Editor.*

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## FEDERAL MILK ORDERS AND THEIR EFFECT ON MILK PROCUREMENT

J. P. MASON

*Division of Economics, National Milk Producers Federation, Washington, D. C.*

Federal orders affect milk procurement to the extent that supplies for individual markets are responsive to changes in relative prices paid producers, as determined on the basis of a use classification. This classification and the pooling provisions are the backbone of federal milk orders, and they are designed to fit the market structure.

Since federal orders regulate milk prices on the basis of the individual marketing area, a certain amount of conflict of interest is unavoidable between regulated markets, and between regulated and unregulated handlers. There is also a divergence of interests between groups of producers who regularly supply a market and those who are potential sources of supply. The latter is true with or without federal orders, wherever dairy farmers are striving to improve incomes by reaching out for better markets.

Other than in the response of milk supplies to price, federal orders are not designed to affect the procurement of milk. They are often cited as the cause of difficulties in marketing, which would not be eliminated by cancelling the orders.

A few marketing situations which are dealt with in federal orders, however, may affect the procurement of milk—pool plant requirements, the allocation of milk, compensatory payments, base-rating plans, the Class I price level, and the excess price level.

### Pool Plant Requirements

Federal orders may provide for individual handler pools or for market-wide pools. The latter are the more numerous, and necessarily require a definition of the market supply, or of pool plant requirements. Under what circumstances should a given supply of milk be absorbed in a pool? Under what conditions should such milk enter the market, if it is not pooled? These decisions must be reached by the Department of Agriculture, and they may affect procurement for individual milk plants. They may influence the decision of a milk plant in deciding whether or not to seek outlets in a federal order market.

Pool plant requirements have been subject to criticism—on the one hand, for not allowing pool participation to plants which ship small amounts of milk into an order market; on the other hand, for allowing pool participation on a too liberal basis.

It is important to remember that milk pools are established by federal orders. Without the orders, there would be few market-wide pools. To the extent that market-wide pools exist in nonregulated markets, participation in them is highly restricted by their nature.

Some plants seek regulation and pool participation; others avoid it. Potential suppliers often refuse to deliver milk to a regulated handler, if it means full regulation for them while their competitors outside the marketing

area are not regulated. In other cases, supplemental supplies may be available to one of several markets, depending upon the prices offered. Health department permits can not be relied upon to determine a milk plant's affinity to a market, since sanitary inspection practices, too, have changed with the newer trends in the industry. Thus, it would be unreasonable to pool milk on any basis other than performance.

Pool participation is normally associated with full regulation. The allocation provisions and compensatory payments serve partially to regulate supplemental suppliers, allowing free entry without total regulation.

The reasonableness of the pool plant requirements is a matter of careful judgment, in light of operating experience. These requirements are subject to change with the needs of the market, but will be necessary as long as the individual marketing area approach to milk regulation is maintained.

## Allocation of Milk

If a regulated handler receives milk both from pool sources and from nonpool sources, some sort of allocation procedure is necessary. Without allocation provisions, it would be impossible to properly classify and price the milk received from producers. Milk may be allocated in any one of a number of ways. In most federal orders, producer milk is allocated first to the higher-priced use classifications, and other source milk to the lower-priced use classifications. Suppliers of supplemental milk often complain that this system discriminates against them.

In practice, regulated handlers purchase milk supplies from producers. When the supply is insufficient, they purchase additional quantities from other sources. In the purchase of supplemental supplies, the price is arrived at competitively, without regard to the use made of the milk by the purchasing handler. Consequently, the allocation of such milk does not affect the price paid to the supplier.

Since federal orders are designed to price milk to producers who furnish the regular supply, it would seem logical that the producer milk should have the advantage of the higher-priced use classification, to the extent that it is available.

If there is a sufficient supply of milk from producers, a handler has no occasion to seek other supplies, and there is no problem. If the market is short of milk and additional supplies are needed for Class I purposes, then the allocation of supplemental milk would be at the Class I use. Producers are paid only for the milk they deliver. Furthermore, no handler is obligated to purchase milk from producers. It can all come from beyond the regular supply and thus receive the Class I allocation. Competitively, however, producer milk normally is the least expensive.

If all regulated handlers are treated the same, and if they have equal opportunity to procure milk from regular producers or from outside sources, there can be no claim of discrimination.

Handlers sometimes make the point that, although they may agree with this position, it cannot be justified during months when a shortage condition exists during part of the month and a surplus condition during another part of the month, resulting in an inflated allocation of producer milk to the Class I utilization. The effects of the allocation provisions cannot be disputed under these circumstances, but the provisions are not inequitable. Any gains to producers that may result from the higher utilization can be considered in establishing the Class I price, or in the determination of resale price schedules.

## Compensatory Payments

Compensatory payments are sometimes referred to as roadblocks to sales in a regulated market. But if a marketing area is to be regulated, it is fundamental that all milk entering the market either conform to the regulation, or be specifically exempt. There must be safeguards in the exemption, to maintain the classified price structure and to assure regulated handlers that their marketing area competitors do not have the advantage of lower-cost milk supplies.

If supplemental milk were allowed to enter a market without regulation, the classified price structure would fall. Any price received in excess of the value of milk for manufacturing purposes may induce an outside plant to ship into a regulated market. But if handlers in a regulated market were permitted to buy milk for Class I use, at manufacturing milk prices from unregulated sources, it would be just as logical to permit them to use the excess milk already existing in the market. If this were permitted, outside shippers would lose outlets, and fluid milk producers would soon find that they were selling all of their milk at the manufacturing milk price.

The alternative course would be to regulate any handler who shipped a pound of milk into an order market. But supply plants would not subject themselves to full regulation while their competitors did not. Such an approach could only serve to deprive suppliers of the supplemental milk of outlets, and limit the sources of supplies for needed milk in a regulated market.

As the orders are administered, compensatory payments are not applied between regulated markets, since each market employs use classification. Compensatory payments applied to milk from unregulated sources are the minimum necessary to assure handlers that their competitors do not have the advantage of lower-cost supplies for Class I uses.

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## Base-Rating Plans

Base-rating plans are in effect in many federal order markets. Although base-rating plans are often thought of as methods of restricting production, or restricting the entry of new producers, the Department of Agriculture does not permit this type of operation.

Base-rating plans serve the single purpose of leveling production. Under federal orders, each producer establishes a new base each year and, for that reason, any adverse effects that the plan may have on the procurement of milk is of relatively short duration.

## The Class I Price

Class I prices established in federal order markets are often cited as responsible for artificially high uniform prices, and for the creation of unnecessary production. We can find no statistical evidence to support this claim.

An examination of milk production, by regions and by markets, will disclose that the production trends have been remarkably similar among the fluid milk markets—whether federally regulated or not—and that production in the manufacturing milk areas has kept pace with that of the fluid milk markets. Furthermore, prices paid for Class I milk in federally regulated markets tend to be somewhat lower than those paid in nonfederally regulated markets and the prices, for the most part, are lower than the cost of available alternative supplies, when transportation is considered.

## The Excess Price

The prices of excess milk in federal order markets are sometimes considered by manufacturing areas as being too low, thus affording the manufacturer of pool milk a cost advantage over the unregulated manufacturing plant. The claim is that the pool handler has the advantage of being able to offer to producers the market blend price—which is higher than that of the unregulated manufacturing plant—for milk which costs less than its competitive value.

It would be difficult to state that the relative cost position of regulated and unregulated plants was at all times in balance. It does appear, however, that the Department of Agriculture has done a reasonably good job in pricing excess milk. The problem is not simple. For one thing, unregulated plants engaged in the manufacture of milk products do not pay uniform prices. There are wide variations in any local area, and the same company will frequently pay widely varying prices for milk used for the same purpose in different localities.

Overriding the price consideration is the need to find an outlet for the excess milk. Standby manufacturing facilities by their nature are operated at a fraction of capacity. In other instances, milk is transported long distances to manufacturing outlets. Excess milk, if sold through regularly established manufacturing facilities, often hits the market at the very time of year that such plants are receiving peak supplies from regular sources. If they are to operate above capacity, this also affects the operating efficiency. In such event, plants tend to pay lower prices for extra volume.

As part of the milk price problem, there is also the relationship between the excess price and the relative profitableness of manufacturing milk as compared to that of the bottled milk business. Many producer groups take the position that the excess price should be at that level which will permit manufacturing operations to break even. It is argued that a profitable manufacturing operation will stimulate procurement for milk that may not be available when needed for fluid purposes. Most manufacturing plants under regulation take the view that the operation is entitled to a reasonable profit and, unless the manufacturing plants accept the milk, market demoralization can result, since the order does not require anyone to purchase the milk.

## Summary

Federal orders cannot reconcile every variance between regulated markets, or between regulated and unregulated handlers, in the procurement of milk. Furthermore, federal order regulations are designed in the best interests of the market and of the dairy industry nationally. They cannot be altered to coincide with the needs of individual milk plants. Milk orders should be kept as simple as practically possible, and maximum freedom of operations should be afforded the handler—provided it accomplishes the goal of the Marketing Act, in establishing minimum prices to producers and in preserving the integrity of the use classification and pooling system.

Federal orders are designed to affect the procurement of milk through use classification and price. It is not intended that the provisions should otherwise afford advantages in procurement to any group of producers or handlers.

Producers supplying fluid milk markets have largely determined that regulation is necessary. If such plans are to be fully effective, it is essential that all milk entering the market conform to the regulation, or that it be exempt on the basis of safeguards which will protect the use classification system.

There have been many criticisms of specific provisions of individual orders, but few complaints of the authority embodied in the Act, or of the administration of the entire program.

No one could argue that errors in judgment have not been made in drafting or administering federal orders. But they have made a tremendous contribution toward the establishment of orderly marketing conditions in an unorderly industry.

# IMPACT OF FOOD AND DRUG DEFINITIONS AND STANDARDS OF IDENTITY ON DAIRY PRODUCTS MANUFACTURE

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The effect of existing federal regulation pertaining to foods preferably should be considered in the light of historical legal precedent *(2)*. The 1906 Act was developed over a period of 25 years, beginning about 1880. About 103 bills, of which 19 were key issues, were submitted in various Congresses, terminating in the Heyburn bill enacted in 1906. This bill served as the law for over 30 years for several reasons: *(a)* There was need for a uniform law in the legal supervision of foods; *(b)* there was need for a law against various forms of adulteration and misbranding, for reasons of fair trade and for public health, *(c)* there was need for such federal law, in the light of the relatively limited supervision over foods in a number of states.

The ultimate development and adoption of the 1906 Act required a long period for a number of reasons, among which were the strong political movements for protection of states rights, and the implication that the philosophy of *caveat emptor* should still prevail. The proposal of the 1906 Act had the support of the food manufacturing and related industries of the times.

There were three major amendments to the 1906 Act which were of significance in the handling of foods. These dealt principally with the definition of food, the labeling of count and weight of certain packaged foods, and the authorization of standards for canned foods.

The 1938 Food, Drug and Cosmetic Act had as principal objectives the establishment of the integrity of foods and the limiting of their debasement, cheapening, or economic adulteration. The 1938 Act differs from the 1906 Act in several distinctive points, among which may be cited the following: *(1)* It prohibits traffic in foods which may be injurious to health, in contrast to the previous provision which prohibited injurious food only when a poisonous substance was added; *(2)* it specifically prohibits the addition of poisons to foods, with defined exceptions; *(3)* it specifically requires label declaration of the presence of artificial coloring, artificial flavoring, and chemicals present in foods, but specifically exempts butter, cheese, and ice cream from the requirement on artificial coloring; *(4)* it provides for the promulgation of definition and standards of identity, and standards of fill of containers for foods, with certain exceptions for fresh and dried fruits and vegetables. Butter was exempted from this provision also, but the Act retains its statutory definition and standard enacted in 1923; *(5)* it requires that labels for foods for which no definition or standards of identity have been promulgated bear the common or usual name of the food, and if made of two or more ingredients, the common or usual name of these, excepting spices, flavorings, and colors which need not be specifically so-named; *(6)* it requires that labels for foods for specific dietary uses inform the purchaser fully of their vitamin, mineral, and other dietary properties; *(7)* the Act does not contain the "distinctive name" proviso of the 1906 Act, through which mixtures or compounds escaped legal control, *(8)* it specifically prohibits the presence of filthy, putrid, or decomposed material, and prohibits insanitary conditions whereby the food may or might be subject to contamination.

## Standards of Identity

Since the adoption of the Food, Drug and Cosmetic Act in 1938, there have been a number of developments and actions which have had effects on, and which have implications for, the dairy industry. The establishment of the standards of identity procedure had several objectives: the enabling of fair trade of common foods, the establishment of integrity, and of consumer understanding of foods. It contemplates not only a specification of the ingredients that may be used, but also where practicable, their quantitative limits. The development of such standards has undoubtedly simplified enforcement procedures. It has unquestionably delimited competition of wholesome with debased foods, and in this respect, allowed the consumer to be assured of the wholesomeness of an intended purchase. The establishment of standards of identity has, in some instances, been a painful and costly experience for both government and industry, requiring expenditures of effort sometimes over periods of years. Having been established, thus painfully, there generally has been great reluctance to propose subsequent changes, in the light of new technical process developments or new ingredient components. It is generally believed that the scope and intensity of research and development on new features of standardized food products are scanty, in comparison with those on nonstandardized foods. A proposed change in a standardized food product may involve a large developmental expense. Experimental marketing of a modified standardized food is subject to permit approval of the Food and Drug Administration, to which all facts must be submitted.

A successful development in the foregoing, ultimately must be followed by hearings for

amendment of the standard, wherein the proponent must reveal in greater part the technical experience acquired and defrayed. The question can be raised then whether the establishment of standards does not in effect stifle new developments in well-established foods. In retrospect, one of the earliest standards developed within the 1938 Act was for evaporated milk. Although a number of experimental modifications have been considered, none has been submitted for consideration for change of the standard.

Two developments in this area are of current interest: One is in the development and marketing of an evaporated canned milk product, in which vegetable fats have been substituted for butterfat. (Such a product has been federally excluded through a prior Filled Milk Act. At present, its manufacture and distribution is limited statewise. Within a number of states, the protective effect of the federal act is greatly diminished when the product is distributed.) A second is the recent development and marketing of concentrated milks, which have many potentialities of form and application. The standards for butter were originally provided by statute in 1923 and included in the 1938 Act. Although modification of butter to produce a wholesome, nutritious, and less costly new product seems technically feasible, apparently no change, or proposal for change, of the standard has been made since the original Act.

The fixity of standards for dairy products must be considered in the light of court appraisal of the 1938 Act. One of the early examples of recognition of the restrictions of a standard is that of the farina case. There had been established through the regular procedure of promulgation two standards of identity for farina: one, a regular farina, excluding all modification except certain vitamins and minerals; the other, an enriched farina, requiring specific B-group vitamins and iron and, permitting optionally, vitamin D. The Quaker Oats Company was denied the right to market the regular farina with added D, not provided for in the standard, although it was labeled as regular farina enriched with added vitamin D. The trial evidence showed that diets of infants were deficient in vitamin D. The administration held that consumers might be unable to make a rational choice of modified forms of the product, even though the product was properly labeled. The Supreme Court ruled that the 1938 Food and Drug Act was not limited to a requirement of truthful or uniform labeling. The court proposed that the remedy lies not in labeling but rather in the authorization of the administration, to promulgate definitions under which integrity of the product can be effectively maintained. It cited further that a common or usual name, such as farina, in this case, would be lost if permission were granted to modify it at will. In effect, the court held that the administration had authority to exclude a disclosed nonharmful ingredient. In effect, the addition of a nonprescribed agent to a standardized food was prohibited, unless the addition resulted in a different kind of food, which could be sold under its own name.

It should not be construed that the existence of standards per se is without benefit. Consider, for example, the problem of distinguishing various types of cheese. Following a lengthy hearing in 1947, standards were issued for some 36 varieties of cheese, 8 general classes of cheese, and 16 categories of process cheese and related items. The development of these standards has aided in defining composition limits, and established what may be considered essential in the art and technology of these products. In the development of such standards, it was necessary, with the cooperation of industry, to unravel area or individual concepts of product. The prevailing practice in manufacture of certain types of cheese in one area may be different from that in another. This may be owing to the effect of local state regulatory experience, or prior apprentice training.

Standards of identity have not yet been established for ice cream. Public hearings lasting almost two years were held for such standards, beginning five years ago. Principal issues are whether the surface-active agents of the polyhydric alcohol type should be recognized as an ingredient, what the standard should be for ice milk, and what should be the minimum required proportion of butterfat in ice cream. Surface-active agents were a major problem in the development of bread standards, and became a subject of newspaper, as well as trade press, debate.

The standard of skimmilk powder was established by statute in 1941, in the definition of nonfat dry milk. Modification of this product to incorporate new developments probably would not be easily achieved, except by Congressional action. Among possible modifications may be cited, alteration of fat and addition of emulsifier and vitamin components.

A function of a standard of identity is to prevent economic adulteration of food as through, lessening the quantities of valuable constituents, the addition of cheaper constituents, or consumer confusion by multiplicity of similar products. The import of such economic adulteration has previously been recognized by Congress through enactment of the Butter Standard Act, the Filled Milk Act, the Oleomargarine Tax Act, and the Filled Cheese Act. A standard for oleomargarine was developed shortly after the enactment of the 1938 Act. It authorized the use of artificial flavor and coloring with appropriate label declaration; the Butter Act of 1923 specifically authorized the use of added color without label declaration. An issue was made that oleomargarine was an imitation for the natural food, butter. A Circuit Court ruled oleomargarine as having an identity of its own, so recognized by the consumer. The

status of oleomargarine was subsequently enhanced by the Oleo Act of 1950. In this, oleomargarine was considered a burden on interstate commerce, whether in inter- or intra-state movement.

Attention might be called, parenthetically, to the observation that, whereas salt is considered an optional ingredient in the standard for butter, salted and unsalted butter are considered by the consumer as separate, different, and noninterchangeable commodities.

## Aspects of Branding

Still another aspect of the 1938 Act involves the standards of identity, but deals primarily with the problem of misbranding. The prior development of a standard of identity for a commodity had been interpreted as precluding, in the light of the farina case, the manufacture and offering for sale of foods made of the same ingredients as provided for in the standard, but of different composition. This position was contested in the celebrated jam case, wherein a manufacturer made and offered for sale, properly identified as Imitation Jam, a product using the same ingredients as provided for in the standards for jam, but of different composition. The court stated the product, properly labeled "Imitation," did not *purport* to be a true jam and thus did not violate the Act. The court held further that the product was legally provided for, having been properly labeled, and was, in fact, a different product. This decision has implications for many dairy products, since they are subject to convenient modification by varying of composition of ingredients cited in existing standards.

The modification of composition of defined products is sometimes done in other ways to escape provisions of the Act. A product consisting of 79% butterfat, plus water, sugar, and vanilla, and suitable for use in ice cream mix has been, and presumably is being, imported into the country under a distinctive name-label. The modification of composition is used to circumvent import quotas on defined products.

The 1950 Act relating to the sale and advertising of oleomargarine delegates to the Federal Trade Commission responsibility for delimiting representation of this product in any way whatsoever as a dairy product. Margarine, manufactured by firms processing meat products, is under jurisdiction of the Department of Agriculture, through the Packers and Stockyards Act, rather than under the Federal Trade Commission, and the effectiveness of the delimiting procedure has been severely questioned; reappraisal is now in suit in Congress.

## Adulteration

An important part of the 1938 Act deals with the aspect or definition of adulteration. Virtually every part of the section of the Act dealing with this definition is under current, intense scrutiny. Shortly after the enactment of this law, the administration instigated prosecution of a creamery company, alleging both the presence of filth in butter and that it had been packed under conditions where it *may* have become contaminated with filth. The decision supporting this allegation of adulteration is an outstanding example of shift in philosophy for responsibility of hazard in a food. Previously, the legal attitude of *caveat emptor* was common, but changed in the current Act to the legal concept that the consumer has every right to assume that even the chance of hazard is not involved in the product.

One part of the Act identifying an adulterated food stipulates it is adulterated if it bears or contains any poisonous or deleterious substance which *may* render it injurious to health. This has been interpreted administratively to mean that if a substance is per se toxic in any degree, it is not allowed in a food—unless it can be shown to be unavoidably present, as for sprays, for which tolerances must be established. This interpretation is commonly called the "per se" doctrine. In application, it requires that any new proposed chemical must be proved harmless. This interpretation has been the subject of much industry, administrative, and legislative scrutiny during the past five years.

In 1950, the Delaney Committee of Congress held over 50 hearings throughout the country to ascertain the extent, and need, for use of chemicals in food production and manufacture. The Committee report indicated that over 700 chemicals were used in foods, but failed to point out that many of these already were provided for in standards, and that many were household items. It resulted in extensive newspaper and magazine publicity, much of which was, by implication, unfavorable to industry. Subsequently, the Food Protection Committee of the National Research Council undertook an independent study of the use of added chemicals in foods, including the amounts used and their functional purpose. This report, covering a study of three years, was published in bulletin form, and lists some 500 chemicals in use.

A similar study is now being made by the Committee, on the subject of incidental additives through use of packaging materials, of which there are many categories. The Committee has made special study of specific categories of food additives on which statements and reports have been issued. Among the Committee reports *(1)* may be cited the following: The Use of Chemical Additives in Food Processing; Safe Use of Chemical Additives in Foods; Principles and Procedures for Evaluating Safety of Intentional Additives in Foods; Safety of Artificial Sweeteners for Use in Foods; Safety of Polyoxyethylene Stearates for Use as Intentional Additives in Foods, and Safety of Mono and Diglycerides for Use as Intentional Additives in Foods.

The Delaney Committee report served to focus attention on the necessity of using newly developed, highly toxic insect and fungicide poisons in order to develop and salvage food crops. Subsequently, Congress enacted the Pesticide Amendment to the 1938 Act, providing recognition of the functional need of agricultural poisons, and directing that tolerances for them be administratively established for the various crops. Several hundred tolerances have thus far been established. The pesticide amendment has at least three features which bear upon the "per se" doctrine of chemical additives. By statute, it recognizes functional necessity of the chemical, where properly established by the U. S. Department of Agriculture. It directs an administrative establishment of tolerances for the additive, derived under the conditions of use of the food and, further, allows the use of a reference scientific advisory committee, in event of differences with administrative decisions.

Another result of the Delaney Committee review, as well as that of a Civilian Committee, was to emphasize the extremely slow and costly procedure involved in proposed modification of existing standards of identity. Congress enacted in 1954 the Hale Amendment to the 1938 Act, providing a new procedure for prompt consideration of proposals for amendment of standards, and their adoption within 90 days, in absence of objections. The Hale amendment provides a means for prompt consideration of incorporation of new chemicals or ingredients, where objection is not anticipated. It is believed this will minimize the restrictive effect of standards on product development, as previously cited.

The "per se" doctrine is interpreted to mean absence of harmful effects. There is much disagreement on the meaning of this term; administratively, it is meant "per se." Industrially, it is proposed it should mean "as of the conditions of use." Administratively, it is held to be essential as per se, since the 1938 Act does not provide to government a mandatory forehand knowledge of the use of chemicals by processors, and requires the administrators to seek and prove the presence of chemicals in foods, and their toxicity—a long and very costly venture.

The definition of toxicity alone has been the subject of considerable debate and dispute. Under the necessity of directive to protect public health, the administration has chosen to consider toxicity in terms of possible cumulative effect over a human life-span. It is recognized this is difficult, if not impossible, because of variations of diet, habit, intensity of consumption, age, and numerous other factors. The extrapolation of biological tests also is not without difficulty, since this involves levels of feeding, methods of introduction into the system, susceptibility of species, criteria for examination, variant of dosage as against common usage, and statistical interpretation.

The enactment of the Miller Pesticide Amendment has resulted in more people giving greater thought to the necessity of pretesting chemicals which are under consideration for use in foods. It has resulted in greater presentation, by industry, to the administration for prior consideration, of evidence concerning the biological properties of chemicals. This, in turn, has narrowed disagreement of the role and function of chemicals, and increased general understanding of the related public health problem. Because of the possible great number of biological procedures that might be involved in the appraisal of the hazard of a chemical in a food, the Food and Drug Administration has published suggested procedures for evaluation of hazard of chemicals.

During the past two sessions of Congress, nearly two dozen bills have been submitted involving appraisal and conditions of use of chemicals in foods. Major features incorporated in the various proposals include the following: *(a)* A grandfather clause, wherein the definition of a chemical additive exempts those substances previously sanctioned for use in the 1938 Act or the Meat Inspection Act, and any substance generally recognized—by experts—through scientific procedure or prolonged use, to be safe under the conditions of intended use; *(b)* that substances having a poisonous nature be required to have functional value; *(c)* that in case of necessity for review of decision, reference be allowed to an ad hoc scientific advisory committee, and to judicial appeal; that the gauge of safety be clearly stated; *(d)* that complete disclosure be made to Food and Drug Administration in advance of plans for sale of foods containing new chemical additives, and *(e)* that proposing users of new chemicals furnish supporting evidence to Food and Drug Administration which shows the material to be safe under the conditions of use of the food.

In the light of the foregoing aspects of the 1938 Act, it is desirable in summary to point attention to some specific related problems within the dairy industry.

In the survey on the use of chemical agents by the Food Protection Committee, it was observed that many processors take for granted the acceptance for safe use of many special functional, compounded products such as emulsifiers, stabilizers, colors, etc. While these are readily identified by brand-name or function, too little is known about the components of these products by many directors of operations.

The Miller Pesticide Amendment has resulted in greatly extended use of powerful insecticides and related agricultural chemicals, which can have carry-over effects through soil, or forage fodder, into milk and dairy products. While tolerances exist for such pesticides in fresh and processed fruits and vegetables, none exists for them in milk and its products. The extensive use of antibiotics of various types, in animal feeds, and as inhibitors of bacterial infections

has, in a similar manner, created problems of appraisal of the degree of hazard which exists when they are present in dairy products. There is a notable difference in the appraisal of the degree of hazard of additives or adulterants in foods, and in dairy products, by various government agencies. This has resulted in confusion of the intent of the regulations, and in industry liability.

A confusing, limiting, and difficult problem for the dairy industry is the frequent great difference between the standards of the respective states and the 1938 Act. While there is a trend in adoption by states of the principles and standards promulgated in the Act, there still exists also strong effort to retain distinctive individual state regulations. The number of dairy processing plants is decreasing. They now cover greater areas, more often engage in interstate traffic, and more often encounter varying regulations for given products.

There is a fine point in the distinction between dairy foods, and products, in the 1938 Act. Thus, filled milk, which is condensed skimmilk and vegetable fat, is subject to the Filled Milk Act; whereas, a proprietary formula of condensed skimmilk, vegetable fat, and vitamins is subject to proprietary or dietary food portions of the Act.

There are many areas in which improvement of nutritional, economic, or quality value of dairy products may be achieved by use of selected functional additives, such as stabilizer, antioxidants, antimycotic agents, flavor protectors or improvers, but which are not used because of lack of legal provision for them, either in federal or state standards. The Food and Drug Administration is empowered to grant temporary permit for test incorporation of a new ingredient in standardized foods. If done without permit, there would apply the *"purports to be"* clause of the 1938 Act, indicating misbranding. The permit procedure is a useful tool, and might well be used more extensively by industry collectively, in achieving desired modifications in existing standards. There is great need for similar procedure within state regulations. It has been used in the tests of hydrogen peroxide and benzoyl peroxide in cheese; its use had led to permissive procedures with chlorotetracycline and oxytetracycline in fresh poultry and in test use for fish.

The Federal Food and Drug Act has been a tremendous force in enabling safe foods, with reduced hazard to general health. Proposed amendments can have far-reaching effects on potentialities for new and modified dairy products, and these should be studied aggressively by members of the dairy industry.

REFERENCES

(1) Academy of Science, National Research Council, Food Protection Committee, 2101 Constitution Avenue, Washington, D. C.

(2) Food, Drug and Cosmetic Law Quarterly. (Reference was made to various issues of the Quarterly.)

## INSPECTION AND GRADING OF DAIRY PRODUCTS

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The Secretary of Agriculture is authorized, under the Agricultural Marketing Act of 1946, to develop and improve standards of quality, condition, quantity, grade, and packaging; to recommend and demonstrate such standards, in order to encourage uniformity and consistency; to inspect, certify, and identify the class, quality, quantity, and condition of agricultural products, including dairy products, under such rules and regulations as the Secretary of Agriculture may prescribe—including assessment and collection of such fees as will reasonably cover the cost of the service rendered.

This action is undertaken with the purpose that agricultural products may be marketed to the best advantage, that trading may be facilitated, and that consumers may be able to obtain the quality of product they desire. The service is operated on a voluntary basis and performed upon request of an applicant. Responsibility for conduct of the grading service has been delegated to the Dairy Division of the Agricultural Marketing Service. Regulations Governing the Grading, Inspection, Sampling, Grade Labeling, and Supervision of Packaging of Butter, Cheese, and Other Manufactured or Processed Dairy Products, identified as Service and Regulatory Announcement No. 169, promulgated by the Department, are available to interested parties.

The purpose of the Agricultural Marketing Service's grading program is to provide an impartial and uniform system of grading and inspection of dairy products, for use by the dairy industry, federal, state, and city governments, and others interested in the sale or procurement of such products on a grade basis. These nationwide programs are carried out under cooperative agreements with state departments of agriculture and colleges of agriculture. This plan of

operation encourages interest and cooperation of state agencies in promoting and focusing attention on quality improvement and grading of dairy products.

The functions of operating the dairy products inspection and grading service are assigned to the Inspection and Grading Branch, with headquarters at Washington, D. C. Immediate supervision of the work is assigned to five area supervisors, located in New York, Chicago, Kansas City, Minneapolis, and San Francisco. The chemist-in-charge of the Dairy Division Laboratory at Chicago is immediately responsible for technical supervision of laboratory personnel at Chicago and other locations where such personnel is utilized. The actual grading and inspection of dairy products is performed by federal employees, except in states where the cooperating state agency provides employees who may be qualified and licensed by the Agricultural Marketing Service for this work.

The minimum entrance qualifications of licensees include at least four years of practical experience in processing or manufacturing dairy products, or a college degree with a major in dairy manufacturing, plus one year of practical experience in a dairy manufacturing plant. Such selected personnel are given intensive training and specialize in uniform application of the U. S. standards for grades of dairy products. Supervision consists of periodic checks on each licensee's work, grading schools and technical discussions relating to all aspects of the grading and inspection programs. The service is self-supporting and operated on fees collected for work performed.

The basis for inspection of dairy products includes U. S. Standards for Grades of Butter, Cheddar Cheese, Swiss Cheese, Dry Whole Milk, Nonfat Dry Milk, Dry Buttermilk, and Dry Whey, Federal Specifications, Military Specifications, and other contract specifications between buyer and seller. Inspection service is available on practically all manufactured dairy products. Inspection and grading generally include quality, quantity, and condition of the products.

Inspection and grading service, also laboratory testing on a lot basis, is available nationwide at various locations, including manufacturing plants, shipping points, assembling points, warehouses, terminal markets, and seaports.

Resident grading service is provided at manufacturing or processing plants on a contract basis. Such plants operate under "USDA Minimum Specifications for Approved Plants Manufacturing, Processing, and Packaging Dairy Products." Under this arrangement, a licensed grader is assigned to the plant full-time. His duties include assistance with quality control, through laboratory testing, checking on sanitary practices and processing procedures, and grading of the finished products.

Processing or repackaging of products owned by the Commodity Credit Corporation (CCC) is performed under the supervision of Inspection and Grading Branch licensed personnel. This work is generally on a contract basis, at the expense of the processing-packaging firm.

Regrading of CCC-owned storage stocks of dairy products also is performed by this Branch. Results of regrading provide valuable information in regard to the stability of the products produced in dairy plants located throughout the United States, and for control and movement of the storage stocks.

Under the provisions of the Regulations, SRA-169, and the current specifications for purchase of dairy products under the price support program, LD-21, plants manufacturing dairy products are subject to inspection. Plant inspections are based on the "USDA Minimum Specifications for Dairy Plants" and include a check on premises, buildings, equipment, facilities, sanitary practices, processing procedures, quality of raw material, and packaging of finished products. When plants are found on inspection to be using unsatisfactory manufacturing practices, equipment or facilities, or unwholesome raw material, or to be operating under insanitary condtions; or if the product is of illegal composition or lacks stability, the plants are placed on an ineligible or probationary status for grading service. Such plants are required to improve their operations before they are returned to full status for grading service. During the calendar year 1956, this Branch performed plant inspections as follows:

| Plant status | Butter | Cheddar Cheese | Dry Milk |
|---|---|---|---|
| Full | 149 | 79 | 276 |
| Probationary | 113 | 138 | 176 |
| Ineligible | 77 | 53 | 73 |
| Total | 339 | 270 | 525 |

Of the 1,134 plant inspections, it is significant that 203 plants were assigned an ineligible status. This situation points up the need for more adequate inspections and control at plant level. Most frequent trouble spots include lack of proper sanitation, lack of grading program on raw material, faulty processing methods, or insect infestation of plant or storage rooms. As we review the impact on the dairy industry after several years of plant inspection work, the thing that stands out most prominently is the tremendous improvement that has been made in the operations at hundreds of dairy manufacturing plants. Buildings, equipment, and facilities have been improved; grading of raw material has been initiated at many plants; better processing methods have been adopted; proper salt treatment of bulk package liners has largely eliminated surface mold on bulk

butter; cabinet and carpet beetle infestation in dry milk plants has been reduced to a minimum, and cheese mites are gradually being brought under control in the nation's cheese factories. The results of the plant inspection work are most encouraging. The need for continued periodic and systematic plant inspection is evidenced by the results of our 1956 plant inspection work. We acknowledge and greatly appreciate the assistance of many state cooperating agencies in carrying this work forward.

For the calendar year 1956, the Inspection and Grading Branch graded, regraded, and supervised repackaging of products in the following volume:

| Product | Commercial | Federal (mostly regrading) | Repackaged for CCC | Total |
|---|---|---|---|---|
| Butter | 646,377,658 | 69,543,920 | 223,234,878 | 939,156,456 |
| Cheese | 232,825,392 | 746,796,137 | 271,300,588 | 1,250,922,117 |
| Dry Milk | 797,495,609 | 75,632,075 | 288,604,405 | 1,161,732,087 |
| Evaporated Milk | 17,527,107 | 8,709,048 | —— | 26,236,155 |
| Miscellaneous | 11,968,885 | 46,692,335 | —— | 58,661,220 |

Percentage-wise, commercial gradings covered approximately 45% of the butter, 23% of the Cheddar cheese, and 54% of the dry milk produced in this country.

For the calendar year 1956, 135,373,728 lb. of butter were labelled with U. S. grade identification. This amounted to 9.61% of the total butter production for the year. This program is increasing rapidly, with considerable interest being shown by retail stores and milk distributors.

We are glad to have had this opportunity to discuss the program operations of the Inspection and Grading Branch.

## GRADING, STANDARDS, AND IDENTIFICATION OF DAIRY PRODUCTS IN FOREIGN COUNTRIES AND THEIR EFFECT ON UNITED STATES EXPORTS AND WORLD TRADE

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The United States is now engaged in world trade in dairy products and, consequently, should be interested in international dairy organizations and their activities. The International Dairy Federation is such an organization. Its activities include an interest in international standards for dairy products. It is to the United States' interest to keep abreast of these activities, in order to help determine and standardize the standards for quality, nomenclature, and the methods of analysis and of inspection and grading of dairy products.

For instance, it would certainly be most helpful to have world-wide uniformity in grading butter. Butter, the most important dairy product in world trade, is universally similar in composition requirements. The butterfat content is usually not less than 80% and moisture requirement not more than 16%. The United States has only a requirement for butterfat content. The United Kingdom, the Netherlands, Germany, Sweden, and France have only a moisture requirement; whereas, Canada, Australia, New Zealand, Denmark, and Luxembourg have requirements for both butterfat and moisture.

There is no uniformity in grade nomenclature. In some countries superlatives are used for the better grades of butter. The United Kingdom has its "extra selected" and "selected"; Australia its "choicest quality" and "first quality," and New Zealand its "finest grade" and "first grade." In Sweden, the "rune" mark is permitted only on butter having "excellent," "very good," and "good" characteristics. In the United States, the federal grading system brings about a similar effect, with its grade "AA" and grade "A" butter. These superlatives have a tendency to defeat the purpose of grading, the second-grade butter being frequently mistaken for the best butter produced. Only those experienced in butter-grading would expect butter to grade higher than "selected," "first quality," or "Grade A."

Specific grade nomenclatures are used by some countries. Canada has "first," "second," "third," and "below third-grade butter"; Germany has Grades "A," "B," and "farm butter"; the Netherlands has Grades 1, 2, 3, and 4, with Grade 1 subdivided into 1 minus, 1, and 1 plus; and Luxembourg has first-, second-, and third-class butter. Denmark has its "Lur" brand and France and the United States may have numerically expressed grades.

Two systems are used to determine the quality grades of butter: *(1)* A score-card system,

where numerical values are placed on flavor and other quality items and then added to obtain the total score or grade. *(2)* A "flavor" system, where the grade of the butter is its flavor score, unless defects in other quality factors lower its grade.

The score system is used in Australia, Canada, France, Germany, Luxembourg, New Zealand, United Kingdom, and the United States. In each of these countries, flavor is the most important item on the score card. Its value ranges from 45 to 70% of the total allowed score. The average value is 50%. Second in importance is the texture of the butter. Its value on the score card ranges from 10 to 30% of the total allowed score, with an average slightly under 22%. Other quality items vary in importance and nomenclature, and all items do not appear on each score card.

The flavor system is used in Denmark, Holland, Sweden, and the United States. Where this system is used, flavor plays an even greater role in determining the grade of butter than it does with the score-card system.

Uniform butter grades and grading would be especially advantageous in world trade. They would permit direct comparisons of the quality and price of butter from different countries. Such uniformity could be readily obtained by cooperative grading and a coordination of grading systems by the butter-producing countries. The systems in use require the same procedure of examining, tasting, and smelling of the butter. They are in general agreement as to the value of flavor. The top qualities of butter in each of the countries studied are of similar quality.

# THE PUBLIC HEALTH SERVICE MILK SANITATION ACTIVITIES IN RELATION TO STATE AND LOCAL PROGRAMS

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The objective of the milk sanitation program of the U. S. Public Health Service is the protection of the public health through the prevention and control of milk-borne disease. However, since protection of the public health is primarily the function of state and local governments, except when specific problems of an interstate nature are involved, this objective is carried out through provision of technical assistance to the states, in the development and conduct of effective milk-sanitation programs. Thus, our activities are basically nonregulatory in nature.

Public Health Service interest in milk, as a factor in the spread of disease, dates back to 1896 *(2)*. During the period 1906 to 1921, comprehensive studies were conducted on the relationship of milk to disease, on the thermal death-points of pathogenic microorganisms in milk, and on the efficiency of commercial pasteurization equipment *(8, 9, 11)*. These early studies led to the conclusion that sanitary control of production, followed by effective pasteurization, was essential for the prevention of milk-borne disease outbreaks; and in 1923 an Office of Milk Investigations was established by the Service, to assist the states in the development of effective milk-control programs at both state and municipal levels.

## Current Program

The current milk-sanitation program of the Public Health Service covers the fields of fluid milk, fluid milk products, frozen desserts, and dry milks intended for use in the reconstitution or manufacture of fluid milk and milk products. Program aims are to encourage the establishment of effective and well-balanced programs in each state; to encourage the adoption of adequate milk-sanitation standards by states and municipalities, and the uniform application thereof; to provide technical assistance to state and local agencies and to industry on problems and new developments which have public health significance; to encourage industry support of, and day-to-day compliance with, state and local milk sanitation programs, and to stimulate increased consumption of pasteurized milk and milk products as a positive public health measure.

To implement these aims, the Service engages in a number of coordinated and related activities which can be grouped broadly into six major categories, as follows: *(1)* Development of recommended sanitation standards, technical procedures, and other program guides; *(2)* conduct of research and investigations; *(3)* provision of technical and advisory assistance to

states, municipalities, and industry; *(4)* education and training; *(5)* development of sanitary criteria for the design and construction of dairy equipment, and *(6)* interstate program responsibilities. In carrying out these activities, which are discussed below, the Service acts in an advisory, consultative, and research capacity, except on those matters concerned with our interstate program responsibilities.

*Development of program guides.* The development of program guides for the use of interested states and municipalities is a fundamental part of our program. The several guides published to date include model ordinances and codes for fluid milk and fluid milk products, and for frozen desserts; model inspection forms, and a method by which states can evaluate the effectiveness of community milk-sanitation programs. Currently under development are recommended sanitation practices for the processing of dry milks used in the reconstitution or manufacture of Grade "A" fluid milk or milk products.

Perhaps the best known of these program guides is the *Milk Ordinance and Code (6)*. Its preparation was an outgrowth of experience gained in assisting the State of Alabama, in 1923, in the planning of a state-wide milk-sanitation program. A study of the differences among state and municipal milk regulations in force at that time, led to the conclusion that a uniform approach was necessary for the control of milk-borne disease, and also would be fairer to the industry. Therefore, to provide assistance to those states and municipalities interested in initiating milk-sanitation programs, the Service developed and published in 1924 a standard milk ordinance. To provide for uniform interpretation of this ordinance, an accompanying code was published in 1927. The code set forth the public health reason for each requirement, and provided details as to satisfactory compliance. It was hoped that by unifying the interpretation of the ordinance, more effective enforcement would result, and that misunderstandings between regulatory agencies and industry would be minimized. Since that time, this recommended standard has been revised 12 times, to keep abreast of modern techniques and new procedures in dairy science, public health, veterinary medicine, and related fields.

The *Milk Ordinance and Code* is not a product of the Public Health Service alone. From the first edition to the present one, the Service has utilized a developmental procedure intended to insure public health adequacy and nationwide applicability. Each edition is developed with the aid of a national advisory committee, whose members include public health and industry representatives as well as experts in the field of dairy science. Research findings, technical developments and changes recommended by official agencies, industry, and others, are carefully reviewed and evaluated. The advice and assistance of the U. S. Department of Agriculture, the Food and Drug Administration, industry organizations, and educational institutions are solicited on specific problems. After these steps have been taken, a draft is prepared which is circulated for review and comment to state and federal agencies, selected municipalities, industry groups and others concerned, and all comments are carefully considered and acted upon before completion of the new edition. This same technique is followed in the development of other recommended standards and program guides.

*Research and investigations.* The conduct of research, the development and testing of new methods and procedures, and the evaluation of the public health significance of new processes, techniques, and equipment, are also a fundamental part of our program. Initially, most of the Service milk-sanitation research effort was devoted to investigation of the efficiency of pasteurization equipment, and to the development of technical procedures, that could be included in the recommended *Milk Ordinance and Code*. The results of this work led, or contributed, to improvements in the design and efficiency of commercial pasteurization equipment, including the development of automatic controls and other safety devices to insure proper pasteurization; establishment of an adequate time and temperature standard for high-temperature, short-time pasteurization, and the establishment of procedures and standards for the bactericidal treatment of dairy equipment.

Studies completed in recent years include, staining procedures for the microscopic examination of milk; the effect of inhibitors in natural waters on the germicidal action of quaternary ammonia compounds; the germicidal properties of chloramine-T, quaternary ammonium compounds, chloramelamine, iodine, and bromine, and investigation of the design and operation of home pasteurization units.

Current research includes two projects of considerable interest, in view of present developments. The first of these involves studies of measurements of flow times of milk and water in closed systems, such as high-temperature pasteurization equipment. Improved methodology for accurate measurements of flow time is being developed, including a radioisotope tracer technique. The second project, which was recently initiated, is a pilot study of radioactivity in milk. This project is a part of the over-all Public Health Service continuing radiation surveillance activity, for measurement of amounts of radioactivity in water, air, milk, and other foods *(7)*.

The Service also participates jointly with industry and other groups in cooperative research projects and, as a means of stimulating research, provides grants to educational institutions for research on specific problems. One project, illustrating both of these approaches, is the

recently completed Q-fever pasteurization study conducted at the University of California, Davis, which was a joint project of the University, the dairy industry, and the Public Health Service.

*Technical and advisory assistance to states.* In order to encourage the development and conduct of effective milk-sanitation programs throughout the nation, the Public Health Service provides technical and advisory assistance to the states. Types of assistance provided include: *(1)* Review and evaluation of state and local programs, including laboratory programs; *(2)* investigation of technical problems submitted by states and municipalities; *(3)* in-service training of state and local milk sanitarians; *(4)* equipment investigations; *(5)* temporary assignment of milk-sanitation specialists to assist states and municipalities in the planning and conduct of demonstration projects and field investigations, and *(6)* provision for interpretations of the *Milk Ordinance and Code* and *Frozen Desserts Ordinance and Code.*

*Education and training.* The Service conducts a number of educational and training activities for state and local personnel. Formal training courses for milk sanitarians are held periodically, and a specialized training course in the bacteriological examination of milk and dairy products is offered annually. In addition, assistance is provided state and municipal health departments, and educational institutions, in the planning and conduct of their own training courses; milk-sanitation seminars for regulatory and industry personnel are conducted by our regional milk consultants; in-service training of new personnel is provided upon request, and training films and training guides are developed for state and local use.

*Sanitary design and construction of equipment.* Our interest in the design, construction, and operation of dairy equipment is threefold. First, we are concerned with function; second, with potential hazards to the milk supply that might result from improperly designed equipment, and third, with the cleanability of the equipment.

As regards *function,* we are directly interested only in those items of equipment in which proper performance of intended function is relied upon as a public health safeguard, such as a pasteurizer and its control appurtenances. As regards *design hazards,* we are interested in the elimination of defects in design, construction, operation, or installation that provide a possible means for contamination of the product. Examples of such hazards are improper relative pressures in regenerator sections and faulty valve design, which permit contamination of a pasteurized product. With respect to *cleanability,* our interest relates to the fact that improperly designed and constructed equipment cannot be properly cleaned, thus may constitute a source of contamination for an otherwise wholesome product. In order to provide safeguards covering these three areas of potential hazard, we have developed and included in the *Milk Ordinance and Code* and other suggested standards, detailed specifications for some types of dairy processing equipment, as well as general sanitary design and construction criteria for all equipment having product-contact surfaces.

Since the practical approach to equipment problems is the elimination of defects prior to manufacture, the Service participates jointly with the Sanitary Standards Subcommittee of the Dairy Industry Committee and with the International Association of Milk and Food Sanitarians, in the development of 3A Sanitary Standards for Dairy Equipment. The 3A program is an excellent example of the manner in which industry and government can work closely together for the public good. Through this program the views of health authorities, the processors, and equipment manufacturers all receive thorough and equal consideration. It provides a means whereby, during development of a standard, manufacturers can evaluate proposed sanitary requirements in terms of fabrication problems and costs, and processors can focus attention on practical considerations involved in utilization and operation of the equipment. It also provides a means whereby local health authorities can accept with confidence dairy equipment built in conformance with the standards.

*Interstate responsibilities.* Two Public Health Service milk sanitation activities are interstate in nature. The first of these is the approval of sources of fluid milk and milk products, and of frozen desserts, served on trains, vessels, and airplanes which move interstate. This is a direct statutory responsibility, and enforcement procedures are governed by the Interstate Quarantine Regulations *(1).* To obtain and continue to carry an "Approved" status, sources must comply generally with the applicable provisions of the *Milk Ordinance and Code* and of the *Frozen Desserts Ordinance and Code.* Currently, 590 sources of fluid milk and milk products, and 368 sources of frozen desserts, carry an "Approved" classification. In order to avoid multiple inspection of individual sources, by federal, state, and municipal agencies, the Service, in its conduct of this regulatory function, accepts the results of inspection and laboratory work performed by local health departments, provided that the local health departments have a satisfactory program of supervision and their results are transmitted through, and endorsed by, the state.

The second activity of an interstate nature in which the Service is engaged is the voluntary State-PHS Program for Certification of Interstate Milk Shippers. Our participation in this cooperative program was initiated in 1951, following requests from the Association of State

and Territorial Health Officers and the First National Conference on Interstate Milk Shipments *(3, 4)*. Under this program, inspection and laboratory control of interstate milk supplies are performed by the states and municipalities in which the source of supply is located, using the *Milk Ordinance and Code* and the rating method developed by the Service as uniform criteria for evaluation. The states report to the Service those shippers whose products and plants have been rated by them in accordance with the applicable sanitary requirements, and the Service quarterly publishes a list of the sanitation ratings of such certified shippers, for the information of areas desiring to import milk. However, no shipper's rating is published without his permission. In order to validate the sanitation compliance ratings submitted by the states, the Service periodically spot-checks and evaluates the work of each state participating in the program.

The objective of this cooperative program is to provide a means whereby health authorities in milk-importing areas can accept with confidence milk shipped into their jurisdictions from out-of-state, without the necessity of making their own inspections of distant sources. While not all states and municipalities are willing to participate, considerable progress has been made through this program in facilitating the interstate movement of high-quality milk. The first list of "Sanitation Compliance Ratings of Interstate Milk Shippers" *(5)*, published in 1951, contained the names and ratings of 160 shippers located in 17 states. The current list *(5)* includes 530 shippers located in 33 states and the District of Columbia. According to a recent survey made by Weckel *(10)*, in which an estimated 60 to 70% of the listed shippers responded, in the year 1955 at least 25,000 shipments were made under the program, representing the transfer of more than 697 million lb. of milk. This was an increase of at least 5,000 shipments and 220 million lb. of milk over shipments made in 1954.

## Summary

The milk-sanitation program of the U. S. Public Health Service, which is concerned with the sanitary quality of fluid milk and fluid milk products, and frozen desserts, is described. The objective of the program, which is basically nonregulatory in nature, is to assist the states in the development and conduct of effective programs for the prevention of milk-borne disease. This is accomplished through development of suggested standards and technical procedures; conduct of research and investigations; provision of technical and advisory assistance to states, municipalities, and industry; education and training, and the development of sanitary criteria for the design and construction of dairy equipment. Public Health Service regulatory functions relating to approval of fluid milk and milk products and frozen desserts served on interstate carriers, and the role of the Service in the cooperative State-PHS Program for Certification of Interstate Milk Shippers, are also discussed.

## REFERENCES

(1) Code of Federal Regulations, as Amended. Interstate Quarantine Regulations, Part 72, Title 42, Govt. Prtg. Off., Washington, D. C.

(2) Faulkner, J. D. The Food Sanitation Program of the U. S. Public Health Service. *Modern Sanitation*, 3: 20. 1951.

(3) Rowland, J. L. National Conference on Interstate Milk Shipments. June 10-12, 1952. *J. Milk and Food Technol.*, 16: 89. 1953.

(4) Rowland, J. L. National Conference on Interstate Milk Shipments. June 9-10, 1953. *J. Milk and Food Technol.*, 16: 232. 1953.

(5) U. S. Department of Health, Education and Welfare, Public Health Service. *Sanitation Compliance Ratings of Interstate Milk Shippers.* May 14, 1951, and April 1, 1957.

(6) U. S. Department of Health, Education and Welfare, Public Health Service. *Milk Ordinance and Code—1953 Recommendations of the Public Health Service.* PHS Pub. 229. Govt. Prtg. Off., Washington, D. C. (3rd prtg., 1955.)

(7) U. S. Department of Health, Education and Welfare, Public Health Service. Public statement concerning pilot research program on radioactivity in milk. May 10, 1957.

(8) U. S. Treasury Department, Public Health and Marine-Hospital Service of the United States. *Milk and Its Relation to the Public Health.* Hygienic Lab., *Bull. 56.* Govt. Prtg. Off., Washington, D. C. 1909. (Out of print.)

(9) U. S. Treasury Department, Public Health Service. *Commercial Pasteurization.* Public Health Bull. 147. Govt. Prtg. Off., Washington, D. C. 1925. (Out of print.)

(10) Weckel, K. G. Status of Use of the Program of Conference Agreements in Interstate Shipments of Milk. Unpublished data. Univ. Wisconsin. 1957.

(11) Williams, R. C. *The United States Public Health Service, 1798-1950.* 1st ed., pp. 363-364. Commissioned Officers Association of the U. S. Public Health Service. Bethesda, Md. 1951.

# EFFECT OF GOVERNMENTAL REGULATIONS AND CONTROLS ON MARKETING DAIRY PRODUCTS

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Governmental regulation of some type or other is inevitable in our modern economic society. While every proposed or enacted law purports to be in the public interest, not infrequently the intent of such laws is to use the government to protect some vested interest, instead of the public. Hence, a major problem is to set forth the purposes of legal administration and then to set up standards that will show whether or not such administration is in the public interest.

At present, there are five major types of governmental regulations that affect the dairy industry. These, together with their principal purposes, are discussed briefly.

*(1) Federal milk orders.* The principal purposes of federal regulation are:

*(a)* To insure consumers an adequate supply of high-quality milk. This is done by setting up pricing mechanisms that insure equity to farmers and equity to distributors. Federal orders do not regulate the quality of milk, but accept the standards set up in each market.

*(b)* To substitute orderly marketing in place of the "law of the jungle" that existed before regulation. The fact may well be emphasized that federal orders were not initiated to support milk prices at monopolistic levels.

*(2) Sanitary milk ordinances.* The principal purpose of sanitary milk ordinances is to insure consumers milk of high quality.

*(3) Anti-trust regulation.* The principal purpose of the Sherman Anti-Trust Act of 1890 is to permit free flow of commodities among the different states. The first section of this act provides that "Every contract, combination in the form of trust or otherwise, or conspiracy, in restraint of trade or commerce among the several states, or with foreign nations, is hereby declared to be illegal" *(1)*.

*(4) Government price supports.* The principal purposes of governmental farm price supports are:

*(a)* To raise and stabilize farm income.

*(b)* To improve social welfare.

*(5) State consumer-price control.* The principal purposes of state consumer-price control are:

*(a)* To stabilize incomes of both producers and distributors. It is assumed that the objective of regulated consumer prices is in the public interest.

*(b)* Prevent unfair competition of stores or other low-priced distribution agencies.

*What is the public interest for the dairy industry?* All studies generally available indicate that present milk consumption in this country falls far below the amount recommended by nutritionists. Hence, a major objective of the dairy industry is to encourage ways for expanding milk sales so that they will be more nearly in line with nutritional needs.

*To what extent does price affect per capita sales of milk?* Governmental regulation, including price-support programs and federal and state price control, as well as anti-trust regulation, needs an accurate answer to the above question. While further research is needed to define more accurately the extent to which price influences milk sales, studies already reported indicate that *(2)*:

*(1)* Per capita sales of milk tend to increase with a decrease in price. The extent of the increase tends to vary in different markets, and with the extent to which price decreases are accompanied by widespread newspaper publicity.

*(2)* People with low and medium incomes tend to make more use of low-priced milk than those in the high-income groups.

*Federal milk orders have helped to increase per capita consumption of milk.* A study of 48 federal order markets (Table 1) showed that:

*(1)* Thirty-eight out of the 48 markets studied did not have excessive milk supplies in the shortage month. These markets included 87% of the population in the 48 markets. Only ten markets had an excessive milk supply in the shortage month.

*(2)* On a national basis, federal milk orders have helped to increase the per capita

TABLE 1

*Milk receipts as percentages of milk used in fluid forms, 48 federal order markets, October and November, 1952, 1953, 1954, 1955, and 1956*[a]

| Markets | Percentage that milk receipts were of utilization | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | October | | | | | November | | | | |
| | 1956 | 1955 | 1954 | 1953 | 1952 | 1956 | 1955 | 1954 | 1953 | 1952 |
| Markets over 500,000 population | | | | | | | | | | |
| Puget Sound | 158 | 157 | 165 | 138 | 114 | 152 | 144 | 145 | 142 | 118 |
| Boston | 148 | 168 | 153 | 159 | 151 | 141 | 154 | 137 | 157 | 144 |
| Louisville | 139 | 122 | 125 | 110 | 109 | 137 | 119 | 126 | 119 | 112 |
| New York | 140 | 159 | 147 | 162 | 141 | 135 | 152 | 143 | 138 | 135 |
| Cleveland | 135 | 136 | 136 | 130 | 133 | 137 | 130 | 137 | 134 | 131 |
| Chicago | 133 | 125 | 122 | 135 | 120 | 142 | 128 | 126 | 155 | 131 |
| Kansas City | 133 | 133 | 124 | 121 | 111 | 142 | 140 | 134 | 138 | 117 |
| Detroit | 132 | 130 | 131 | 128 | 125 | 133 | 125 | 129 | 134 | 128 |
| Minneapolis | 128 | 122 | 107 | 118 | 111 | 135 | 125 | 114 | 138 | 128 |
| New Orleans | 126 | 127 | 130 | 120 | 99 | 141 | 122 | 133 | 123 | 113 |
| North Texas | 119 | 105 | 106 | 106 | 96 | 122 | 103 | 105 | 115 | 103 |
| Philadelphia | 118 | 120 | 124 | 112 | 116 | 115 | 119 | 123 | 117 | 114 |
| Milwaukee | 114 | 106 | 107 | 107 | 102 | 115 | 106 | 106 | 118 | 108 |
| Cincinnati | 108 | 111 | 110 | 101 | 112 | 109 | 103 | 109 | 109 | 109 |
| St. Louis | 105 | 94 | 99 | 93 | 82 | 106 | 95 | 102 | 103 | 88 |
| San Antonio | 87 | 81 | 80 | 90 | 79 | 87 | 81 | 78 | 95 | 79 |
| Markets under 500,000 population | | | | | | | | | | |
| Duluth-Superior | 175 | 191 | 164 | 157 | 150 | 183 | 192 | 169 | 163 | 142 |
| Dubuque | 164 | 141 | 130 | 133 | 122 | 176 | 144 | 140 | 156 | 138 |
| Quad Cities[b] | 154 | 139 | 137 | 133 | 108 | 156 | 158 | 138 | 142 | 115 |
| Cedar Rapids-Iowa City | 148 | 146 | 136 | 128 | 110 | 162 | 144 | 135 | 142 | 116 |
| Neosho Valley | 143 | 130 | 128 | 130 | 114 | 150 | 138 | 143 | 152 | 121 |
| Wichita | 141 | 125 | 132 | 127 | 120 | 149 | 136 | 133 | 154 | 132 |
| Topeka | 139 | 123 | 113 | 106 | 97 | 159 | 131 | 128 | 128 | 111 |
| Oklahoma City | 129 | 123 | 119 | — | — | 128 | 120 | 119 | 131 | 110 |
| Muskegon | 124 | 129 | 125 | 120 | — | 125 | 121 | 119 | 129 | — |
| Tulsa-Muskogee | 124 | 123 | 118 | 118 | 101 | 132 | 122 | 119 | 132 | 107 |
| Dayton-Springfield | 118 | 126 | 121 | 109 | 117 | 116 | 121 | 118 | 119 | 115 |
| Fort Smith | 118 | 113 | 99 | 112 | 101 | 130 | 108 | 96 | 124 | 105 |
| South Bend-La Porte | 117 | 107 | 125 | 111 | 98 | 122 | 115 | 118 | 116 | 100 |
| Nashville | 116 | 105 | 112 | 100 | 106 | 132 | 109 | 121 | 112 | 110 |
| Stark Co. | 116 | 120 | 122 | 114 | — | 114 | 117 | 120 | 122 | — |
| Omaha-Lincoln–Council Bluffs | 115 | 119 | 112 | 110 | 87 | 126 | 125 | 115 | 121 | 94 |
| Fort Wayne | 113 | 106 | 153 | 128 | 143 | 107 | 105 | 139 | 132 | 134 |
| Knoxville | 112 | 95 | 94 | 98 | 99 | 120 | 100 | 90 | 104 | 97 |
| Sioux Falls-Mitchell | 112 | 121 | 99 | 105 | 87 | 122 | 128 | 95 | 108 | 99 |
| Columbus | 111 | 113 | 114 | 108 | 107 | 113 | 114 | 118 | 120 | 112 |
| Sioux City | 108 | 113 | 112 | 115 | 99 | 119 | 119 | 112 | 128 | 108 |
| Lima | 107 | 115 | 133 | 122 | 100 | 112 | 114 | 109 | 134 | 104 |
| Memphis | 107 | 105 | 97 | 93 | 97 | 108 | 105 | 100 | 103 | 102 |
| Tri-State | 104 | 97 | 103 | 89 | 87 | 100 | 93 | 95 | 93 | 88 |
| Paducah | 102 | 99 | 101 | 73 | 72 | 99 | 95 | 99 | 77 | 66 |
| Central W. Texas | 96 | 105 | 81 | 96 | — | 102 | 103 | 82 | 95 | — |
| Rockford-Freeport | 95 | 88 | 100 | 110 | 105 | 101 | 91 | 101 | 116 | 115 |
| Springfield, Mass. | 92 | 107 | 112 | 106 | 107 | 94 | 102 | 106 | 111 | 106 |
| Toledo | 90 | 97 | 97 | 94 | 83 | 97 | 101 | 102 | 104 | 89 |
| Fall River | 86 | 99 | 80 | 93 | 97 | 87 | 100 | 73 | 95 | 95 |
| Merrimack | 84 | 107 | 100 | 98 | 99 | 84 | 102 | 94 | 100 | 96 |
| Worcester | 80 | 88 | 89 | 86 | 91 | 81 | 84 | 85 | 89 | 91 |

[a] Adapted from Regulations Affecting the Movement and Merchandising of Milk, USDA Marketing Research Report No. 98, June, 1955, p. 47, Table 18; additional data obtained through the courtesy of J. C. Blum, USDA, in a letter dated April 5, 1957.

[b] Davenport, Iowa; Clinton, Iowa; Rock Island, Illinois, and Moline, Illinois.

consumption of milk and have substituted orderly marketing in place of the law of the jungle *(3)*.

*(3)* Three suggested ways for bringing excessive Class I prices into line, in markets where reserves are too high, are to: *(a)* publicize the facts; *(b)* increase competition by selling concentrated milk or dry whole milk powder produced in low-cost areas in the high-priced markets; and *(c)* introduce remedial legislation, if necessary. Such legislation should not be attempted, however, until after other ways of attaining the desired objective have failed.

*Are sanitary milk ordinances acting as trade barriers to greater milk consumption?* Enforcement of sanitary milk ordinances has improved milk quality and encouraged greater per capita consumption. On the other hand, the writer is inclined to agree with the findings of Dr. A. C. Dahlberg who, with his associates, made a very careful study of the sanitary ordinances in eight markets for the National Research Council. He stated *(4)*:

"Excessive sanitary regulations which serve to restriet the number of producers in a market add heavily to the cost of the product. There is no excuse for this, and it is not desirable for anybody in the long run to continue with high-cost health regulations. We should work to get milk to consumers at the lowest possible cost. The necessary minimum of regulation to protect the public health is what the public requires—and no more."

*Should anti-trust action be extended?* Evidence indicates that there are some sanitary milk ordinances that function as trade barriers to the free intermarket and interstate flow of milk. To the degree that these ordinances are effective in raising prices above their competitive level, they are acting as barriers to increased consumption of milk or cream.

In contrast with this situation is the one found in Chicago. Here, as stated, per capita milk consumption increased 39% between 1940 and 1955, or nearly three times that for the country as a whole (14%). This increase followed the Supreme Court decision of 1939, upholding the indictments of the Anti-Trust Division of the United States Department of Justice *(5)*.

Looking at this situation in perspective, one might well ask: Would not further action by the Anti-Trust Division or by an individual firm be desirable at this time?

Health departments are wise in being cautious about permitting new products to come onto the market without proper safeguards as to quality. However, after reasonable safeguards have been established, one may well question either the economic or legal justification of refusing to permit high-quality products to enter into interstate commerce.

*What about government price supports?* Support prices for dairy products should be established at a level that, over a period of years, will permit a high percentage of milk production to be absorbed by the commercial market—namely, to "clear the market." Under this theory, in some years no government purchases will be necessary—in other years, substantial purchases will be necessary (as they were in 1953, for instance). Under this theory, the major function of the government is that of securing stability of income, not relief.

*Are support prices for nonfat solids too high?* Between 1953 and 1956, 46% of all nonfat solids were purchased by the government in its support program (Table 2). Furthermore, rapid expansion of the industry is now in process. Several new plants for processing nonfat dry milk are being built in Minnesota, and expansion of present facilities is reported for Wisconsin and other producing areas. If support prices are continued at their present level, more and more nonfat dry milk will be produced to be sold to the government for 16¢ or 14.25¢ per lb., and then given away or sold at a low price for animal feed or for other uses.

TABLE 2

*Government purchases of dairy products for price supports*[a]

| Year | Per cent of total production | | | |
|---|---|---|---|---|
| | All milk produced | Butter | Cheese | Nonfat dry solids |
| | (%) | | | |
| 1952 | 0.3 | 1.1 | 2.4 | 6.0 |
| 1953 | 8.3 | 22.3 | 21.6 | 48.4 |
| 1954 | 7.5 | 19.6 | 20.0 | 46.4 |
| 1955 | 3.8 | 10.4 | 11.4 | 38.7 |
| 1956 | 4.3 | 11.1 | 13.4 | 49.3 |
| Average 1953-56 | 5.9 | 16.0 | 16.5 | 45.8 |

[a] USDA.

In view of these facts, it is recommended that consideration be given to lowering the support prices of nonfat dry milk a minimum of 4¢ a lb. below present support levels.

From a long-run viewpoint, such action might be expected to do two things:

*(1)* Slow down the uneconomic expansion in the production of nonfat dry milk.

*(2)* Stimulate the commercial sales of this product.

*Should price supports for cheese be reduced?* If consumer income is held at a high level, it is probable that the present high consumption of meats will be maintained, if not increased,

creating keen competition for cheese in the commercial market. Furthermore, a review of the facts indicates that high support prices for cheese are tending to perpetuate inefficient production of this product. Finally, the facts indicate that the disposal of government purchases of cheese is far more difficult than disposal of butter. Hence, it is recommended that consideration be given to reducing the support price of cheese 20%.

*Are price supports for butter too high?* Marked progress has been made since 1953 in curbing excesses of government purchases of butter and disposing of the volume purchased. Probable increases in per capita consumption of milk within a few years may further reduce government purchases of butter. Hence, at this time it would appear desirable to maintain support prices of butter at their present level.

*Is state control of consumer milk prices in the public interest?* Analysis of this question may be summarized as follows:

*(1)* At the high point in the 1930's, 26 states had laws fixing the minimum prices to be paid by consumers for milk. Together, these states had a population (1950 census) of 113 million, or about three-fourths of the entire population.

*(2)* Fourteen of the 26 states once having state control of consumer prices have eliminated this type of control.

*(3)* The multiplicity of products and sizes of containers on which prices must be fixed greatly complicates the problem facing a state control agency.

*(4)* Enforcement of minimum prices charged to stores, restaurants, and other wholesale outlets is difficult, if not impossible. In some markets secret discounts, rebates, tie-in sales, loans, and gifts have become so general that they are the rule rather than the exception.

*(5)* State control of consumer prices tends to prevent the introduction of new inventions that have proved helpful in competitive areas in increasing per capita consumption of milk.

On the basis of available evidence, the writer is inclined to agree that under present conditions the disadvantages of resale price-fixing outweigh the advantages, and that from a public-interest viewpoint it would be desirable to find ways to eliminate this type of governmental control.

*How does governmental regulation affect the distributor's spread?* A study of distributors' gross handling margins in 145 markets in March, 1957, showed that:

*(1)* The gallon container is the key to low-cost distribution of milk in many markets. In ten of the 15 low-margin markets, two out of three sold milk at stores in gallon jugs (Table 3). This proportion was over three times that for the 145 markets included in this study, where only one of five (30 markets) sold milk at gallon prices.

*(2)* Five markets in the low-margin group sold milk in half-gallon containers. Hence, low distributor margins, as shown by this study, were directly associated with quantity sales at low prices.

*(3)* In federal order markets, keen store competition, which tends to lower distributors' margins, is possible without lowering prices to producers. Ten of the 15 low-margin markets were under federal milk orders.

*(4)* Only one out of 33 markets under state consumer price control was included in the 15 low-spread markets, and this market, Alexandria-Arlington, was just across the river from Washington, D.C., which had the lowest margin. State control of consumer prices tends to keep distributors' margins higher than those in markets with keen store competition.

TABLE 3

*Distributors' gross margins for handling milk in 145 markets, March, 1957* [a]

| | Distributor gross margin | | |
|---|---|---|---|
| | Lowest store price | Av. of all consumer prices reported | Single-quart, home-delivered price |
| | *(per qt.)* | | |
| 15—Market average: lowest | 6.7 | 9.9 | 11.4 |
| 145—Market average | 10.5 | 11.6 | 12.2 |
| 15—Market average: highest | 13.5 | 13.6 | 13.5 |

[a] Data as computed from USDA, Fluid Milk and Cream Report, March, 1957.

*What relation exists between governmental regulation and the development of fresh milk substitutes?* The development of a sterile concentrated milk and a dry whole milk powder, which can be substituted in place of fresh whole milk, is likely to break down all types of trade barriers now obstructing the free flow of milk between the different states.

Recent studies have shown that:

*(1)* At the 1957 Farm and Home Week at the University of Illinois, 35% of those taking the taste test thought sterile concentrated milk (reconstituted) was regular milk. The sterile milk used in the test, processed by Dr. E. O. Herreid, was two months old, and had been held in tin cans at 70° F.

*(2)* One firm that has already invested over half a million dollars in research on sterile concentrate estimates that the market for it will be around six billion quarts annually, or about one-fourth of that for fresh whole milk.

*(3)* Another firm which has developed a sterile concentrate is planning to market it on a national basis within a year.

*(4)* At present, at least 50 scientists in 20 different institutions, including private firms and universities, are engaging in research to develop concentrated milk or whole milk powder that can compete with fresh whole milk.

The development of fresh milk substitutes may well be watched with interest by all groups in the dairy industry.

*Synthetics are displacing cotton.* In 1913, 90% of all fibers used in the United States were cotton. By 1956, this proportion had fallen to 65%.

In 1913, production of synthetic fibers was negligible. By 1955, synthetics constituted 26% of the total amount of fibers used.

The declining domestic consumption of cotton may be directly associated with high support prices, together with increased competition from synthetics.

*The electric industry: Low prices and high volume per consumer.* Residential consumption of electricity increased from an average of 398 kwh. per consumer in 1929, to 2,549 kwh. in 1954, a sixfold increase.

During this same period, the price per kilowatt hour dropped sharply. In 1929, the price averaged 6.33¢ per kwh.; by 1954 it had declined to 2.69¢, or about two-fifths of that for 1929.

The electric industry has operated on the principle of mass production at low unit costs. Both production and service costs have been reduced through volume output and use of new technology.

The dairy industry can profit by the experience of the electric industry.

*Long-time outlook for the dairy industry.* There are both favorable and unfavorable factors now affecting the dairy industry. Among the favorable ones are: *(a)* A steady increase in population; *(b)* a steady increase in purchasing power of consumers; *(c)* an increase in per capita consumption of frozen dairy products and nonfat dry milk solids; *(d)* the development of new low-cost methods for distributing milk; *(e)* an inventive group of scientists whose new products are likely to benefit consumers as well as the dairy industry, *(f)* an amazing increase in the efficiency of producing milk, which is likely to continue.

Among the unfavorable factors may be listed: *(1)* Loss in the per capita consumption of butter and evaporated and condensed milk; *(2)* increasing pressures among producer groups, sometimes encouraged by labor unions, to demand governmental price supports at unduly high levels. In some areas, such as Detroit and New York, violence has been used in an attempt to attain this objective; *(3)* an excessive volume of governmental purchases of nonfat dry solids and cheese resulting from high price supports; *(4)* the cost-price squeeze, which is particularly difficult for those who have just commenced dairying (this is not, however, peculiar to the dairy industry); *(5)* excessive governmental regulation for states under state consumer price control and for a few federal order markets, *(6)* use of sanitary milk ordinances as trade barriers to prevent the intrastate and interstate flow of milk and milk products.

The future of the dairy industry depends to a considerable degree upon the types of governmental policies that are put into operation.

In the first place, will governmental action be taken to prevent sanitary milk ordinances from being used as trade barriers, in the flow of milk within the separate states and between the different states?

In the second place, are state consumer price controls going to continue to prevent the introduction of new technology which, in competitive areas, has resulted in lower-cost milk distribution and lower prices to consumers?

Finally, what is the probable future of governmental price supports for dairy products? If, on the one hand, the advocates of high governmental price supports should succeed in attaining their objective through use of production controls and individual marketing quotas, dairying, like cotton, could become a declining industry. High price supports would tend to lower per capita milk consumption and encourage substitution similar to the increased use of synthetics in the fiber industry.

If, on the other hand, governmental price supports are kept at a level that over the years will clear the market, the dairy industry, like the electric industry, can look forward to a long period of growth and expansion. Under a favorable environment, trade barriers that now exist are likely to be sharply curbed as new products go around them, give consumers more for their money, and give farmers a larger share of the consumer's dollar.

It is certainly to be hoped that the long-time enlightened self-interest of the dairy industry can be its guiding policy, rather than the short-time viewpoint of those who would ignore the history of industries that have already succumbed to the weakening influences of excessive governmental controls, or have failed to use the governmental programs that operate in the public interest.

## REFERENCES

(1) Barriers to Increase Consumption of Fluid Milk. A special report by the National Grange, January, 1955, Chapter 7, pages

104 to 108. Hearing before the special subcommittee to investigate the dairy industry in the United States, of the Committee of Agriculture, House of Representatives, 83rd Congress, Part 3, July 7, 1953, Serial D, page 463, Table 2.

(2) Cornell Agr. Expt. Sta., *Bull.* 765, 1941; Storrs Agr. Expt. Sta., *Bull.* No. 280, July, 1951; Alabama Polytechnic Inst. Agr. Expt. Sta., *Bull.* No. 282, June, 1952; Illinois Farm Economics 203, April, 1952, pp. 1340-1347; *Ibid.* June, 1953, pp. 1484 to 1487; USDA Cit. 645, Mag. 1942; Ohio Agr. Expt. Sta., *Research Cir.* 29, March, 1956.

(3) Governmental Control of the Dairy Industry Other than Health Measures and Standards. *J. Dairy Sci.*, 39: 892. 1956.

(4) Sanitary Milk Control and Its Relation to the Sanitary, Nutritive and Other Qualities of Milk. National Academy of Sciences, National Research Council, Publication 250, 1953.

(5) BARTLETT, ROLAND W., *The Price of Milk.* Interstate Printers and Publishers. pp. 113-124, Chapter 8. Anti-Trust Enforcement. 1941.

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# JOURNAL OF DAIRY SCIENCE

## ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

### BOOK REVIEWS

**566. A Textbook of Dairy Chemistry. Vol. II, Practical.** Third edition revised. EDGAR R. LING. Chapman and Hall Ltd., London. Price, 16 shillings.

This edition forms an excellent basis for a practical, undergraduate laboratory course in Dairy Chemistry. The emphasis is placed on analytical tests and techniques employed in connection with milk and dairy products.

Material covered here, which is not found in the previous edition of this book, includes experiments on the turbidity test involving heat-denatured whey proteins, phosphatase test, detection of quaternary ammonium compounds in milk, detection of reactive sulfhydryl groups in heated milk, action of detergent solutions on hard water, determination of lactic acid, determination of sucrose in sweetened condensed milk, and the determination of residual chlorine in water. In some instances, different tests or modifications of tests used in the earlier edition are presented. There are also a few more illustrations of apparatus and additional discussion. J. Tobias

**567. The Freezing Preservation of Foods. Vol. II—Cooked and Prepared Foods.** 3rd ed., 559 pp. D. K. TRESSLER AND C. F. EVERS. The Avi Publishing Company, Inc., Westport, Conn. 1957.

Volume I of this pair, *Freezing of Fresh Foods*, has been reviewed in this Journal (July, 1957). An extensive review of Volume II is not necessary, because it follows the pattern of the first volume, and the same plaudits apply.

The freezing of ready-to-eat foods, covered in a single chapter in the previous edition, now requires an entire book for adequate coverage. The first chapter justifies this emphasis. Although research on frozen baked goods, prepared poultry, meat dishes, etc., was initiated in 1930, consumer interest in such products was almost completely lacking until the present decade. Now the freezing of ready-to-eat foods is rapidly becoming a separate industry.

Chapter 2, a discussion of special problems encountered in this field, builds on the information given in 9 chapters of Vol. I and is especially good on the microbiology of precooked frozen foods. The material presented on packaging of these products is also well done.

The chapter entitled "Ice cream, Sherberts, and Ices" is probably elementary to most dairy technologists, but the 70 references appended should be helpful. The major value of this book to readers of this Journal would be the extensive coverage given to the many foods, such as cheesecake and candy, which contain dairy products. Formulas and production methods are given for most of the prepared foods. From this standpoint alone this book is worth obtaining.

United States Standards for grades of several frozen prepared foods, as well as a few military specifications, are presented in the Appendix. M. P. Steinberg

**568. Manuale Lactis. 11 Folge, Klasse 6. Milcherzeugnisse, Nebenproducts. (Milk Manual, Series 11, Class 6. Milk Products, By-Products.)** M. E. SCHULZ AND G. SYDOW. Verlag, Hans Carl, Nürnberg 2, Germany.

This is one of a series of handbooks of the dairy industry which began appearing during the years 1948 and 1949. The current one contains a bibliography of articles published in many parts of the world on subjects dealing with dairy products other than milk, butter, and cheese. Both literature and patents appearing during the years 1939 to 1956 are abstracted under the following headings: *(1)* General; *(2)* sour milks, yoghurt, kefir, buttermilk, acidophilus milk; *(3)* milk-fruit drinks, cocoa drinks, malted milk, milk champagne; *(4)* ice cream, frozen milk, frozen cream, freezing techniques; *(5)* condensed milk products, evaporated milk, sweetened condensed milk, sterilization of condensed milk; *(6)* dry milk products, whole milk powder, skimmilk powder, buttermilk powder, cream powder; drying techniques, methods; packaging, utilization of milk powder; special products; *(7)* whey and whey

products; *(8)* specially processed milks, baking agents, egg substitutes; *(9)* foods incorporating milk, such as baked goods, bread, pastries, milk chocolate, binding agents for sausage, substitutes for baking fats, and *(10)* other topics, calculations (by-products without milk and industrial by-products).

For the convenience of English-speaking readers, a reference to an English journal containing the abstract is also made.

J. Tobias

**569. Grundlagen der Chemie, Anorganische Chemie in einfacher Darstellung.** K. Hennies. Hans Carl, Nürnberg, Germany. 455 pp. 18.50 D.M. 1957.

This book deals with basic inorganic chemistry as currently presented in a first-year course in chemistry in the United States. It is very similar in content and arrangement of subject material to *Introductory College Chemistry* by H. N. Holmes, or to *General Chemistry* by Sisler, Vanderwerf, and Davidson.

In an American book a short history of chemistry is usually given as an introductory chapter. In this German counterpart, the history of chemistry is given in perspective, in the last chapter of the book. The remaining chapters are, however, written in orthodox style, with more emphasis on physical chemistry than is usually given in a book serving as an introduction to inorganic chemistry. These chapters include a discussion of the general laws of chemistry, the nonmetals, rare gases, metals, and radioactive elements. The last four chapters contain a short discussion of colloid chemistry.

This book could serve as an excellent means of study for American graduate students who previously have not been exposed to scientific German. It would serve to broaden a background of German as usually given at the high school or undergraduate student level.

F. A. Kummerow

## BUTTER

**570. Cheese and butter slicer.** E. Peckover. U. S. Patent 2,804,686. 2 claims. Sept. 3, 1957. Offic. Gaz. U.S. Pat. Office, **722**, 1:29. 1957.

Individual rectangular-shaped portions of cheese and butter are formed by hand by this device, consisting of a cutting blade and slotted, spatula-like handle. R. Whitaker

**571. Sur la teneur en eau des beurres stockés en chambre froide (Moisture content of cold storage butter).** J. Cusalis, L. Serres, and S. Amarigles. Le Lait, **37**, 370. 1957.

It is concluded that there is practically no moisture loss during cold storage of properly worked butter. There are varying degrees of moisture loss when poorly worked butter is placed in storage. These losses can be serious, but can be overcome by proper working. The latter is the method of control.

R. W. Rudnick, Jr.

## CHEESE

**572. Acidity development in Italian-cheese starter cultures.** W. J. Harper and R. M. Huber, Dept. of Dairy Technol., Ohio State Univ., Columbus. Milk Prod. J., **48**, 8:16. 1957.

Differences in the ratio of the surface area to the depth (S/D) of the media were related to problems involving slow acid development during the manufacture of experimental Provolone cheese. The S/D ratio of the milk in the cheese vat must be similar to that which has been utilized in carrying the starter culture. Failure to utilize S/D ratios similar to that required in the cheese vat resulted in failure in the manufacture of Provolone cheese.

The authors conclude that some difficulties in acid production by apparently active starter cultures may be related to marked differences in surface-depth ratios of the medium in which the cultures are grown, when this problem cannot be related either to bacteriophage or to antibiotics. J. J. Janzen

**573. Hot pack, cream-type cheese from reconstituted milk ingredients.** J. P. Malkames, G. H. Norman, and C. Jason, 13th Air Force Central Exch., APO 74, San Francisco. Milk Prod. J., **48**, 6:36. 1957.

The authors discuss the manufacture of a hot pack, cream-type cheese using low-heat nonfat milk solids, anhydrous butterfat, anhydrous citric acid crystals, salt, and locust bean gum. This cheese possessed a pleasing flavor similar to that of cream cheese. It had a smooth texture and firm body at 40° F., the spreadability was fair to good and improved with increase in temperature.

Detailed manufacturing procedures are presented. The product can be held safely for a period of from 4 to 5 wk. at 40° F. Flavor deterioration starts at about 5 wk.

J. J. Janzen

**574. The tyrosine decarboxylase potential of milk and cheese.** P. Raiband and G. Mocquot, Natl. Inst. for Agr. Research, Jony-en-josas, France, and F. V. Kosikowski, Dept. of Dairy Ind., Cornell Univ., Ithaca, N.Y. Vol. 2, Proc. 14th Internatl. Dairy Congress.

Since tyrosine decarboxylase is not present in cow, goat, or sheep milk, it may be assumed that its presence in cheese is due to microbial origin.

Roquefort, Cantal, Pont l'Eveque, and Carre-de-l'est all showed variable concentrations of tyrosine decarboxylase originating from microbial sources.

The amino acid decarboxylase system, particularly bacterial surface-ripened cheese, would preclude any great decarboxylation of free tyrosine during ripening. The authors believe that a deaminase system would become activated to attack the free tyrosine to form phenolic compounds and ammonia, while other free amino acids would become deaminated to their respective end products and ammonia.

H. H. Weiser

**575. The heat stability of bacterial tyrosine decarboxylase.** P. R. RAIBAND AND G. MOCQUOT, Natl. Inst. for Agr. Research, Jony-en-josas, France, AND F. V. KOSIKOWSKI, Dept of Dairy Ind., Cornell Univ., Ithaca, N.Y. Vol. 2, Proc. 14th Internatl. Dairy Congress.

The heat stability of the tyrosine decarboxylase from *Streptococcus faecalis* R was determined in milk and whey at different pH levels.

Tyrosine decarboxylase added to fresh milk at pH 6.65 was inactivated after heating the milk to 61.8° C. for 30 min., 65.5 °C. for 20 min., and 71.1° C. for 20 sec. Thirty-five per cent of the enzyme remained active when heated to 71.1° C. for 8 min. at pH 5.5. The stability of this enzyme at low pH and high-heat treatment may have some significance of such extreme stability to gas blowing of processed cheese.

No real evidence has been obtained to show that gas blowing of some processed cheese originates from heat-resistant enzymatic sources, or even that the heat stability of the amino acid decarboxylase in cheese is similar to that observed in milk and whey. H. H. Weiser

**576. Some unidentified nitrogenous compounds of cheese.** G. J. SILVERMAN AND F. V. KOSIKOWSKI, Dept of Dairy Ind., Cornell Univ., Ithaca, N. Y. Vol. 2, Proc. 14th Internatl. Dairy Congress.

Refinements in two-dimensional paper chromatography for ripened cheese resulted in a better resolution of nitrogenous components.

The chemical composition of raw and pasteurized milk cheese showed differences in total amino acid concentrations and some nitrogenous compounds. Putrescine, cadaverine, and tyramine are present in raw but not in pasteurized milk cheese. Also, large amounts of serine and low levels of aspartic acid are present in well-ripened pasteurized milk cheese.

Apparently, a portion of the casein molecule, possessing a high proline content, resists certain bacterial proteinase of Cheddar cheese, or it may be that free proline is utilized by microorganisms for nutritional purposes.

H. H. Weiser

**577. Machine for forming cheese balls of uniform weight and shape.** A. A. MONGIELLO AND Z. BILOUS. U.S. Patent 2,803,878. 6 claims. Aug. 27, 1957. Offic. Gaz. U.S. Pat. Office, **721**, 4: 660. 1957.

Spherical portions of cheese are cut from cheese by a hollow semispherical cutting mold and discharged one at a time. The cheese is rotated and pressed against the ball-forming mold by compressed air. R. Whitaker

**578. Knife for cutting cheese and the like.** E. C. NELSON, U.S. Patent 2,803,876. 1 claim. Aug. 27, 1957. Offic. Gaz. U. S. Pat. Office, **721**, 4: 659. 1957.

A description of a knife designed for cutting cheese by hand. R. Whitaker

## CONDENSED AND DRIED MILKS; BY-PRODUCTS

**579. Increasing the protein content of milk products.** E. G. STIMPSON AND H. YOUNG (assignors to National Dairy Products Corp.). U.S. Patent 2,809,113. 15 claims. Oct. 8, 1957. Offic. Gaz. U.S. Pat. Office, **723**, 2: 357. 1957.

Milk or other dairy product, containing 0.05 to 0.06% added available nitrogen, is cultured with a yeast or mold at 80-100° F. and at pH 3.5 to 7.5, to increase the protein and decrease the lactose content. The growth of the microorganisms is facilitated by aeration within the range from about 0.1 to 2.5 volumes of air per volume of medium per minute during incubation. A treatment is given the final product to release the cell contents.

R. Whitaker

**580. Method for the preparation of mashed potatoes.** E. G. COOPER. U.S. Patent 2,802,742. 2 claims. Aug. 13, 1957. Offic. Gaz. U.S. Pat. Office, **721**, 2: 349. 1957.

In the manufacture of mashed potatoes from dehydrated potato powder, 2% of nonfat dried milk solids is added. R. Whitaker

**581. Alginate composition for making milk puddings and process for making the same.** K. F. GIBSEN (assignor to Kelco Co.). U. S. Patent 2,808,337. 21 claims. Oct. 1, 1957. Offic. Gaz. U.S. Pat. Office, **723**, 1: 147. 1957.

A thickening agent for a milk-base pudding consisting of alginic acid, sodium carbonate, and a calcium part. R. Whitaker

**582. Process and apparatus for manufacture of casein.** P. H. SMART AND C. E. NELSON (assignors to Foremost Dairies, Inc.). U. S. Patent 2,807,608. Sept. 24, 1957. Offic. Gaz. U.S. Pat. Office, **722**, 4: 790. 1957.

Alkaline casein is acidified and before precipitation takes place is fed continuously into a flowing stream of acid solution where, on continued movement, it forms a rope-like curd mass. When fully insolubilized, the precipitated casein is directed out of the acid stream for washing and drying. R. Whitaker

**583. Process for cooling, pressing, and drying of protein-containing glue.** M. G. YAFFI.

U. S. Patent 2,807,051. 3 claims. Sept. 24, 1957. Offic. Gaz. U. S. Pat. Office, **722**, 4: 654. 1957.

Hot, wet, coagulated casein is passed between two rolls, which cool it to 16° C. or below, to form a continuous sheet. R. Whitaker

## DAIRY BACTERIOLOGY

**584. A technique to increase sensitivity of the disc assay method for antibiotics in milk.** F. V. Kosikowski, Dept. Dairy Ind., Cornell Univ., Ithaca, N. Y., and G. Mocquot, Natl. Inst. for Agr. Research, Jony-en-josas, France. Vol. 2, Proc. 14th Internatl. Dairy Congress.

The use of circular dried-milk tablets instead of paper discs, used in the standard assay method for antibiotics in milk, increased the sensitivity almost tenfold. It was possible to detect 0.01 I.U. penicillin/ml, 0.1 unit aureomycin/ml, and 0.5 unit dihydrostreptomycin or neomycin/ml in milk.

Apparently, the mechanics of diffusion of the antibiotic down through the dried-milk tablet is a contributing factor of sensitivity.

Greater sensitivity is attained by decreasing the amount of agar on the standard petri dish; however, the thinner agar layer may be uneven and may crack during incubation.

H. H. Weiser

**585. Preparation of cheese cultures.** A. B. Erekson (assignor to the Borden Co.). U. S. Patent 2,805,950. 6 claims. Sept. 10, 1957. Offic. Gaz. U. S. Pat. Office, **722**, 2: 362. 1957.

A starter for Swiss cheese is prepared from a culture of *Streptococcus thermophilus* which is free from lactobacilli, yeasts, and molds. The starter is grown in whey to an acidity of 0.35 to 0.40%; then 5 to 15% by weight of milk powder is added, the mixture cooled and frozen for subsequent use. R. Whitaker

**586. Sur l'emploi d'une bactérie thermophile pour un dosage rapide de la nisine (Use of a thermophilic organism for rapid determination of nisin).** P. Raibaud. Le Lait, 37: 378. 1957.

The author makes use of an organism with optimum growth at 68° C. to determine biologically the amount of nisin present. Advantages claimed for the method are that the medium is simple and does not need to be sterilized, aseptic techniques are not essential, and results are readily determined by pH of the cultures. R. W. Rudnick, Jr.

**587. La recherche d'antibiotiques, seuls ou associés, dans le lait. (Detection of antibiotics, singly or combined, in milk.)** P. Dopter. Le Lait, 37: 20. 1957.

By use of strains of *S. lactis* accustomed to growth in small amounts of various antibiotics, the authors set up a systematic scheme for determining which of several antibiotics are present in milk. They report that the technique can be used by any competent industrial laboratory. R. W. Rudnick, Jr.

## DAIRY CHEMISTRY

**588. Amino acid composition of adult and foetal myoglobin of the cow.** R. Timmer, H. J. van der Helm, and T. H. J. Huisman. Nature, **180**, 4579: 239. 1957.

Myoglobin from these two sources was studied by means of paper electrophoresis and column chromatography. No significant difference was found. Some differences were observed in the hemoglobin from the two sources.

R. Whitaker

**589. Automatic densimeter jar.** G. M. Wallace. Dairy Ind., **22**, 5: 419. 1957.

This instrument is designed to provide a means of determining rapidly the density of milk by means of a lactometer. It consists of a cylinder, provided with a side arm for filling without foam formation, a thermometer well, an overflow giving a constant level, and a drain pet cock. The instrument may be cleaned without removing the lactometer. R. Whitaker

**590. Activity and specificity of rennin.** J. C. Fish. Nature, **180**, 4581: 345. 1957.

The proteolytic activity of rennin has been shown to be specific and not due to contaminants. R. Whitaker

## DAIRY ENGINEERING

**591. Stopping deterioration in recirculating cooling water systems.** P. G. Bird, Wright Chemical Corp., Chicago, Ill. Ind. Refrig., **133**, 1: 15. 1957.

A corrosion-control review, this article contains information concerning both mechanical inhibitors (theory and practical application), common inhibitors, dosage and pH control, and means of controlling solids. It is reported that the three most important control factors are quantity of inhibitor, pH control, and concentration cycles. R. W. Rudnick, Jr.

**592. Operating pointers for cutting refrigeration costs.** W. J. Kampman, Creamery Package Mfg. Co., Chicago, Ill. Ind. Refrig., **133**, 1: 30. 1957.

Acting on the premise that refrigeration is one of the major operations in the dairy plant, and thus a major source of operating and repair expense, the author gives a detailed check list on reducing these expenses. Each item of equipment in the refrigeration system is listed, and precautions noted. R. W. Rudnick, Jr.

**593. Developments in dairy processing equipment.** H. L. Mitten, Jr., Creamery Package Mfg. Co., Chicago, Ill. Milk Prod. J., **48**, 6: 80. 1957.

The author discusses the new and significant developments in the dairy industry, including new larger-capacity equipment, new circulation cleaning equipment, flavor-treating equipment and processes, ultra high-temperature processing equipment, instantizers for dried milk solids, and flow meters.

In addition, a brief discussion is presented on new features of design for existing types of processing equipment, such as positive pumps, paper packaging and overwrap equipment, new cup machines, etc. J. J. Janzen

**594. Bottle pocket.** J. B. McCABE (assignor to Cherry-Burrell Corp.). U. S. Patent 2,804,966. 2 claims. Sept. 3, 1957. Offic. Gaz. U. S. Pat. Office, **722**, 1:100. 1957.

Details are given for the construction of a pocket for holding bottles in a bottle-washer. R. Whitaker

**595. Apparatus for emptying containers of liquid.** W. S. EDWARDS (assignor to National Dairy Products Corp.). U. S. Patent 2,803,361. 4 claims. Aug. 20. 1957. Offic. Gaz. U. S. Pat. Office, **721**, 3:520. 1957.

Paper milk-bottles containing liquid dairy products are emptied by passing them through this device, which crushes them between two grooved rollers, delivering the product to a reservoir and collecting the crushed containers to facilitate disposal. R. Whitaker

**596. Container-handling apparatus.** R. E. PALMER (assignor to Oswego Falls Corp.). U. S. Patent 2,805,753. 4 claims. Sept. 10, 1957. Offic. Gaz. U. S. Pat. Office, **722**, 2:313. 1957.

A conveyor is described for arranging paper milk-bottles, moving in single file, into pairs with selected sides adjacent. R. Whitaker

**597. Refrigerated storage tank.** E. M. STEXEKORN, W. G. ESPERSON, AND J. AMMERMAN (assignors to U. S. Industries, Inc.). U. S. Patent 2,803,952. 5 claims. Aug. 27, 1957. Offic. Gaz. U. S. Pat. Office, **721**, 4:679. 1957.

An insulated, jacketed round-bottom tank is described, having an agitator, flush valve, and refrigerating system. R. Whitaker

**598. Refrigerating tunnel.** A. FRIEDMAN AND D. LURIE (assignors to Joe Lowe Corp.). U. S. Patent 2,808,790. 15 claims. Oct. 8, 1957. Offic. Gaz. U. S. Pat. Office, **723**, 2:273. 1957.

Stick novelties, hanging from a series of stick-holders in a track installed in a low-temperature tunnel, are moved forward to permit rapid hardening prior to packaging. R. Whitaker

**599. Milk-carton crusher.** R. E. PALMER (assignor to Dari Research Corp.). U. S. Patent 2,808,776. 10 claims. Oct. 8, 1957. Offic. Gaz. U. S. Pat. Office, **723**, 2:269. 1957.

A device for removing milk from returned paper cartons. The cartons are squeezed by a roll, the milk drawn to a reservoir, and the crushed cartons impaled on pins and later removed for discharge, separately from the milk. R. Whitaker

**600. Implement for transferring frozen confections from bulk into small containers.** V. B. PHILLIPS, J. G. EVERETT, AND R. HOEKEMA. U. S. Patent 2,808,791. 1 claim. Oct. 8, 1957. Offic. Gaz. U. S. Pat. Office, **723**, 2:273. 1957.

A device for cutting cylindrical-shaped portions of ice cream from bulk cans. The portions are slit lengthwise by a crosswire. R. Whitaker

## DAIRY PLANT MANAGEMENT AND ECONOMICS

**601. The dairy products industry under the amended Federal Wage-Hour Law.** N. BROWN, Administrator, U. S. Dept. of Labor. Milk Prod. J., **48**, 8:18. 1957.

The author reviews the provisions of the Act popularly known as the "Federal Wage-Hour Law," as they may be affected by the new minimum wage law. The following phases are highlighted in the discussion: *(1)* Employee coverage; *(2)* "first processing" exemption; *(3)* overtime pay exemption for outside buyers; *(4)* "white-collar" exemptions; *(5)* motor carrier exemption; *(6)* retail establishment exemption; *(7)* overtime computation; *(8)* child-labor provisions, and *(9)* record-keeping. J. J. Janzen

**602. Ten billions spent in U. S. for dairy products in 1956.** Anon. Ice Cream Rev., **41**, 3:66. 1957.

Data released by the Milk Industry Foundation show that, for the first time, consumers of the United States spent over 10 billion dollars for milk and dairy products in 1956. This figure represents 16.6% of the total food bill in the United States during 1956. Out of every dollar spent for dairy foods in 1956, 13.7 ¢ went to the ice cream industry. W. J. Caulfield

**603. Cut distribution costs.** G. PATTERSON, The Borden Co., New York, N. Y. Ice Cream Rev., **41**, 3:50. 1957.

Three changes were introduced by the Borden Co. to reduce distribution and handling costs of ice cream. The first change was to substitute pallets for conveyors in handling the stock. This change resulted in a saving of from 12 to 15 man-hours in unloading a 30-foot trailer. The second change was to intrdouce a center-aisle, walk-in type route truck with full-flush rear doors. Each truck is equipped with five removable rack sections, which ride on roller conveyor sections built in the truck floor. The third change effected had to do with the

method of loading the route trucks. Under the new system, the truck is backed up to an opening in a vestibule maintained at 45° F. The rear door of the truck is completely open during the loading operation. The load is prepared by rolling the removable racks onto dollies which, in turn, are rolled into the storage area and the order made up. When the rack is loaded, it is returned to the vestibule, checked, and pushed back into the truck. This new method of loading the route trucks has resulted in labor savings of from 40 to 50% over the former method.

In handling individual orders on the route, the driver enters the truck body through the door from the cab and makes up the order. The various items are placed on the floor adjacent to a small service door. The items are removed from the truck through this service door. This new type of truck, and method of handling the stock, are being heartily endorsed by the driver salesmen. W. J. Caulfield

**604. Milk-bottle carrier.** W. J. COMSTOCK. U. S. Patent 2,806,731. 2 claims. Sept. 17, 1957. Offic. Gaz. U. S. Pat. Office, **722**, 3: 569. 1957.

A handle made of wire for carrying a glass milk-bottle. R. Whitaker

**605. Container holder.** R. J. BURNS. U. S. Patent 2,807,399. 3 claims. Sept. 24, 1957. Offic. Gaz. U. S. Pat. Office, **722**, 4: 744.

A wire holder for paper milk-bottles is described. R. Whitaker

**606. Collapsible multiple compartment carton.** K. T. BUTTERY (assignor to Sutherland Paper Co.). U. S. Patent 2,808,177. 5 claims. Oct. 1, 1957. Offic. Gaz. U. S. Pat. Office, **723**, 1: 107. 1957.

A carrier for carrying several bottles of milk. R. Whitaker

**607. Stackable carrying case.** E. C. VOIGHT. U. S. Patent 2,802,589. 6 claims. Aug. 13, 1957. Offic. Gaz. U. S. Pat. Office, **721**, 2: 312. 1957.

A wire case for milk bottles is described. R. Whitaker

## FEEDS AND FEEDING

**608. Method for increasing nutritive value of foliage.** I. J. BELASCO, M. F. GRIBBINS, D. W. KOLTERMAN, AND F. B. VAUGHAN (assignors to E. I. du Pont de Nemours and Co.). U. S. Patent 2.802,307. 5 claims. Aug. 13, 1957. Offic. Gaz. U. S. Pat. Office, **721**, 2: 239. 1957.

To improve the nutritive value of roughage for ruminants the plants are sprayed with an aqueous solution of urea 8 hr. to ten days prior to harvesting. R. Whitaker

**609. Process for feeding ruminants and improved feed supplement therefor.** P. C. ANDERSON AND J. L. C. RAPP (assignors to Feed Service Corp.) U.S. Patent 2,808,332. 21 claims. Oct. 1, 1957. Offic. Gaz. U. S. Pat. Office, **723**, 1: 147. 1957.

The amount of nitrogen retained by ruminants from a feed is increased by the addition of a source of synthetical nitrogen and some ethyl alcohol. R. Whitaker

**610. The effect of the duration of whole milk feeding on the growth of dairy calves.** R. L. STEELE[1], Dept. of Dairy Husb., W. Va. Univ., Morgantown.

Sixteen dairy calves of Jersey, Ayrshire, and Holstein breeds were randomly assigned to two treatments and compared to see if any differences could be detected in growth and digestive disorders under two different durations of whole milk feeding. Calves under the two treatments were managed and fed alike, except for the duration of the milk feeding. The one group of calves received 399 lb. of milk per calf from birth to 8 wk. of age; the other group received the same amount of milk per calf, but from birth to 12 wk. of age. Hay and starter were fed free choice.

Calves in both treatments were above normal in growth after 8 wk. of age and there was no significant difference in weight. However, at 20 wk. of age there was a difference of 30 lb. per calf in favor of the calves which received milk for 12 wk., which approached statistical significance. This group of calves also required less total digestible nutrients per pound of gain for the 16-wk. feed-recording period. They required only 2.18 lb. of total digestible nutrients per pound of gain, as compared to 2.28 lb. of total digestible nutrients per pound of gain required by the calves receiving milk for 8 wk. R. L. Steele

[1] Prepared from a portion of a thesis submitted by the author in partial fulfillment of the requirements for the M.S. degree at West Virginia University.

## GENETICS AND BREEDING

**611. Genetics of the $\beta$-lactoglobulins of cow's milk.** R. ASCHAFFENBURG AND J. DREWRY. Nature, **180**, 4582: 376. 1957

Two different $\beta$-lactalbumins, A (formerly $\beta_1$) and B (formerly $\beta_2$), were determined electrophoretically in the milk of 278 cows. From the data presented, support is given to the hypothesis that the formation of these proteins is controlled by a single gene. The authors deduced the type of gene of the bulls from previous offspring. A possible similarity of the $\beta$-lactoglobulin content of milk to the blood groups of the cattle is suggested. It also appears likely that there is a relation between the concentration of each of the lactalbumins and the amount of casein in the milk and that the two

lactalbumins differ in the lactalbumin: casein ratio. The physiological significance of this is briefly discussed. R. Whitaker

## HERD MANAGEMENT

**612. Milking systems.** H. H. Hill. U. S. Patent 2,805,643. 2 claims. Sept. 10, 1957. Offic. Gaz. U. S. Pat. Office, **722**, 2: 284. 1957.

A pipeline milker is described. The system has a single pulsating vacuum line leading from a single vacuum pump to ten or more milking units. The milk from each unit is automatically discharged to a reservoir and then into a single milk line, for delivery to a storage tank. R. Whitaker

**613. Barn-cleaning apparatus.** G. B. Carlson, A. O. Carlson, and A. E. Carlson. U. S. Patent 2,804,194. 3 claims. Aug. 27, 1957. Offic. Gaz. U. S. Pat. Office, **721**, 4: 743. 1927.

Waste material in a barn gutter is pushed to the end of the gutter by an endless flexible conveyor, equipped with logs which act to scrape the walls of the gutter. The conveyor, in turn, is cleaned by a rotating brush. R. Whitaker

**614. Milking and collecting apparatus.** R. R. Graves (assignor to Graves-Stambaugh Corp.) U. S. Patent 2,808,025. 22 claims. Oct. 1, 1957. Offic. Gaz. U. S. Pat. Office, **723**, 1: 67. 1957.

An arrangement of a pipe-line milker which prevents the milk from coming in contact with air while it is weighed and discharged through a pipe line. R. Whitaker

**615. Milk-can cover.** R. M. Harris. U. S. Patent 2,808,959. 3 claims. Oct. 8, 1957. Offic. Gaz. U. S. Pat. Office, **723**, 2: 317. 1957.

A milk-can lid with an expanding band, which seals the can tightly when a toggle linkage arrangement is operated by hand to expand the band. R. Whitaker

## ICE CREAM

**616. Guard device for frozen confection.** J. M. Ackalusky. U. S. Patent 2,803,550. 1 claim. Aug. 20, 1957. Offic. Gaz. U. S. Pat. Office, **721**, 3: 568. 1957.

A device for holding a stick-novelty type of frozen confection, consisting of a tray with side walls for catching the drip from the confection as it melts. R. Whitaker

**617. Automatic ice cream freezers.** L. C. Updegraff. U. S. Patent 2,808,706. 15 claims. Oct. 8, 1957. Offic. Gaz. U. S. Pat. Office, **723**, 2: 249. 1957.

A small freezer which operates in conjunction with a home food-freezing cabinet. R. Whitaker

**618. Ice cream freezer.** H. H. Bruderlin. U. S. Patent 2,806,679. 8 claims. Sept. 17, 1957. Offic. Gaz. U. S. Pat. Office, **722**, 3: 555. 1957.

A small hand-operated ice cream freezer is described. R. Whitaker

## MILK AND CREAM

**619. Factors that affect the stability of frozen concentrated milk.** R. W. Bell, Dairy Products Section, Eastern Utilization Research and Development Division, USDA. Milk Prod. J., **48**, 8: 12. 1957.

The problem of the preservation of concentrated whole and skimmilk in the frozen state can be divided into two parts; namely, the retention of a satisfactory body and an acceptable flavor.

Body retention is dependent on the physical stability of the casein complex. Factors affecting stability of the proteins include the degree of concentration, heat treatment, homogenization, and rate of freezing.

The development of oxidized flavor can be prevented or slowed considerably by heat treatments and/or the addition of ascorbic acid. Concentrated milk is more resistant to the onset of oxidized flavor during frozen storage than is unconcentrated milk. However, old, stale flavors and a loss of flavor character are frequent defects.

While the retention of both flavor and body is of prime importance, a 40% solids frozen concentrate has a strong tendency toward gel formation. The preservation of a satisfactory body, rather than flavor, becomes the limiting factor.

Several graphs are presented showing the stability of normal and multiple solids milk at various storage temperatures, and the influence of enzymatic lactose hydrolysis on the protein stability of concentrated whole milk. J. J. Janzen

## NUTRITIVE VALUES OF DAIRY PRODUCTS

**620. Gastric digestion of homogenized milk in school children.** E. Czech, Berlin. Monatsschr. Kinderh., **105**, 88. 1957. (From abstract in J. Am. Med. Assoc., **164**, 12, 1398. 1957.)

Ten children, 14 to 18 yr. of age, were given 300 cc. of cow's milk, together with a 7% mixture of lactose and dextrose, on an empty stomach. On consecutive mornings, homogenized milk and nonhomogenized milk were used in the observations. Stomach-tube samples were taken at 30-min. intervals. Homogenized milk remained in the stomach a shorter time. The differences varied from 30 to 150 min., average 80 min. The data indicated that less gastric juice is required for the digestion of homogenized milk. The author concludes that homoge-

nized milk is more susceptible to the action of digestive enzymes, because of the disintegration of the fat and because of the softer coagulation of the protein. Frank E. Rice

**621. Influence of L-lysine supplements on the absorption and retention of nitrogen from milk by children with protein malnutrition. Preliminary report.** F. GOMEZ, R. RAMOS-GALVAN, J. CRAVIOTO, S. FRENK, C. DE LA PENA, M. E. MORENO, AND M. E. VILLA, Hospital Infantil, Mexico City. J. Pediat., **51**, 3: 262. 1957.

Five male children, aged 16 to 36 mo., suffering from chronic severe malnutrition, were placed on metabolic beds for the collection of urine and feces, separately. Cow's milk was offered, to provide 100 to 150 calories per kilogram of actual body weight. Three levels of L-lysine were added to the basal diet—100, 250, and 500 mg. per meal. Each child was fed five times per day. For each feeding period, total nitrogen absorption is reported (difference between total intake and fecal excretion) as well as "balance" (intake minus total excretion). High absorption and retention values were observed in all periods. Lysine supplements were practically without effect. It is concluded that the biological value of cow's-milk protein is adequate for malnourished infants, and that supplementation with the amino acid, lysine, is unnecessary.

Frank E. Rice

**622. The pathogenesis of idiopathic hypercalcemia in infancy.** T. STAPLETON, W. B. MACDONALD, AND R. LIGHTWOOD, St. Mary's Hospital Medical School, London. Am. J. Clin. Nutrition, **5**, 5: 533. 1957.

All the infants reported as suffering from idiopathic hypercalcemia had been fed on formulas prepared from cow's milk. The authors believe that this may be one of the factors that predispose to the development of the disease. While the serum calcium level is high in this condition, serum phosphorus is usually in the normal range. The disease is more common in the United Kingdom than in North America. The authors discuss the possibility that the predominant use of dried milks in the former area, as against the more common use of evaporated milk in the latter, may be a factor. High vitamin D intake appears to be a conditioning factor. It is believed that in European countries babies are given more highly fortified vitamin D foods and vitamin supplements than in America. The authors discuss inconclusively the possible relation to the disease of dietary-essential fatty acids. (Abstractors note: The observations reported here are in strange contrast to the beliefs held in some medical circles —that cow's milk–formula feeding predisposes an infant to *hypo*calcemia, with attendant tetany.) Frank E. Rice

**623. Unsaturated-fat feedings and serum cholesterol levels in infancy.** J. POMERANZE AND A. GOALWIN, New York. Am. J. Diseases Children, **94**, 4: 447. 1957.

Reported here in a preliminary way is the beginning of studies designed to help clarify the problem of atherogenesis and lipid metabolism, especially in early life. Serum cholesterol levels were determined on: *(1)* normal infants from birth to 2 yr. of age (and compared with data reported from countries where infant-feeding practices are different) *(2)* infants fed foods containing predominantly unsaturated fats, and *(3)* six infants to whose evaporated-milk formulas 50 g. of corn oil was alternately added and removed at 3-wk. intervals.

In normal infants, on the usual evaporated-milk formula, serum cholesterol rises during the first 6 mo. from an average of 80 mg. to over 150 mg. per 100 cc. In Italian infants, the rise has been reported to be significantly less. In infants fed substituted milk products in which vegetable fats replace animal fats, serum cholesterol levels do not rise. Serum cholesterol levels fall when 50 g. of corn oil is added to the evaporated milk formula, and rise in the absence of corn oil. The authors state that their studies thus far do not completely answer the questions in this area of study. Results of their further researches may be expected. Frank E. Rice

**624. Intake of linoleic acid in relation to serum unsaturated fatty acids and fat-deficiency symptoms in infants.** A. E. HANSEN, H. F. WIESE, D. J. D. ADAM, A. N. BOELSCHE, AND M. E. HAGGARD, Galveston, Texas. Am. J. Diseases Children, **94**, 4: 398. 1957.

Human milk contains four to five times the amount of linoleic acid that is present in the usual cow's-milk formula fed to infants. It is noted also that there has been a tendency in recent years to feed babies on special products containing even less milk fat than that contained in the usual formula prepared from whole cow's-milk. In this study, five different milk formulas were supplied by an infant-food manufacturer, all having the same composition with respect to calories, protein, minerals, and vitamins, but differing in the kind of fat and the amount of linoleic acid in the mixtures. One of the mixtures contained neither fat nor linoleic acid; another contained only saturated fats. Almost 170 infants were maintained for periods up to 9 mo. on one of the five mixtures.

In the two groups of babies fed mixtures containing no fat and only saturated fats, the serum values were extremely low for two and four double-bond fatty acids (linoleic and arachidonic), but high for a three double-bond fatty acid. Clinical findings associated with the skin and intestinal tract of a large proportion

of the babies in these two groups made it necessary to change feeding procedures. Blood serum levels and clinical symptoms were corrected by the administration of approximately 2% of the calories as linoleic or arachidonic, as well as by shifting to a milk mixture containing unsaturated fatty acids. The authors conclude that linoleic acid is essential in the feeding of the young of the human race as well as in animal nutrition. Frank E. Rice

**625. Metabolic studies of infants fed pasteurized and fresh human milk.** S. J. Fomon and C. D. May, Univ. Iowa Med. School, Iowa City. Am. J. Diseases Children, **94**, 4: 396. 1957.

This is a digest of a paper presented at the annual meeting of the American Pediatric Society, together with a record of questions and comments from members of the audience following the presentation.

Six normal infants were fed human milk by bottle, all or part of the first 6 mo. of life. The human milk used had been pooled, pasteurized, frozen, and stored. It was fed ad libitum, supplemented by vitamins A, C, and D. No other foods or accessory food substances were given. Three-day nitrogen balance determinations were made on each baby about every 2 wk. Nitrogen intakes at one month, which were 351 mg/kg/day, gradually decreased to 232 mg. at 6 mo. Mean nitrogen retention of 170 mg/kg/day at 1 mo. decreased to 50 mg. per day at 6 mo. A check was made by feeding unprocessed human milk for a period of time, with no significant differences in results. It was concluded, therefore, that the processing operation did not change the nutritive value of the milk.

The authors point out that the intakes of nitrogen and the retentions reported here are considerably less than have been observed with babies on cow's-milk formulas. Inasmuch as these human milk–fed babies "progressed along normal growth channels," the authors assume that larger nitrogen retentions, such as are obtained through cow's-milk formula feedings, are unnecessary and even may be harmful. They point out the need for more satisfactory criteria for evaluating the advantages or disadvantages of feeding babies at protein levels higher than those provided by human milk.

Frank E. Rice

**626. Prematurity and rickets.** S. Eek, L. H. Gabrielsen, and S. Halvorsen, University Pediatric Clinic, Rikshospitalet, Oslo, Norway. Pediatrics, **20**, 1: 63. 1957.

In this study, 69 prematures were divided into three groups and fed as follows: *(1)* human milk from a breast-milk bank; *(2)* cow's milk diluted with an equal volume of water, with 7% added sugar during the first 8 wk. and later on, two parts of cow's milk to one part of water with 5% sugar, and *(3)* human milk modified by adding 2% of dry skimmilk. Vitamins C and D were given to all groups, and an amino acid preparation was added to the diets of Groups 1 and 2 to bring the protein content of these diets up to that of Group 3. Calcium, phosphorus, and phosphatase were determined in the serum. Clinical and radiological examinations were made as indications of bone development.

Serum calcium in Groups 1 and 2 was about equal, but was significantly lower in Group 3. Serum inorganic phosphorus was lowest in Group 1 and highest in Group 2, the differences increasing with age. Phosphatase values increased from the neonatal level, with the greatest increase in Group 1, particularly from 60 to 100 days. Before that time, serum phosphatase was higher in Group 3 than in Group 2 and about the same as in Group 1. Although no rickets was observed in any groups, on several occasions inorganic serum phosphorus levels of the babies on human milk corresponded to values usually found in active rickets.

The mean daily growth in length of infants fed human milk was 0.104 cm., as compared with 0.116 cm. for the infants fed cow's milk. However, this difference was not statistically significant. Certain bone abnormalities, such as craniotabes, were more frequently seen in the infants on human milk. No correlation could be established between disease or diet of the mother during pregnancy and any of the biochemical, radiologic, or clinical findings in the infants. The authors conclude that cow's-milk feedings give better mineralization than do human-milk feedings. Frank E. Rice

**627. Intake of vitamin $B_6$ and infantile convulsions: A first approximation of requirements of pyridoxine in infants.** O. A. Bessey, D. J. D. Adam, and A. E. Hansen, Depts. of Biochem. and Nutrition, and Pediatrics, Univ. of Texas, Medical Branch, Galveston. Pediatries, **20**, 1: 33. 1957.

This is a report of observations on 20 infants with convulsive seizures. Five of these infants had been fed a proprietary product (SMA), later found to be low in vitamin $B_6$. These babies had been receiving from this product less than 0.1 mg. of $B_6$ daily for from 2 to 4 mo. prior to the time the convulsions appeared. When transferred to evaporated-milk formulas (providing an estimated 0.26 mg. vitamin $B_6$ daily), the babies experienced no further convulsions.

Two infants with convulsions had been breast-fed. One was changed to an evaporated-milk formula, which again brought about a remission of symptoms. The administration of pyridoxine to the other gave a similar response. The breast milk which one of these infants had been receiving was found to be providing the baby with only 0.067 mg. $B_6$ per day. Two babies in the study were found to require large doses

of $B_6$ to prevent the appearance of convulsive seizures; in one case, medication was required even to the age of 20 mo. These cases were apparently due to a metabolic aberration that led to an unusually high requirement for the vitamin, and had no relation to dietary deficiency.

Eight hospitalized infants with no history of convulsions, who had received an evaporated-milk formula, served as control subjects for the biochemical studies. The tryptophane-load test was performad on a number of babies as a measure of $B_6$ requirement. The correlation was not absolute between the amount of vitamin $B_6$ required to prevent an excretion of xanthurenic acid in this test and the intake required to prevent convulsive seizures. As a result of all observations made, the authors conclude that on a vitamin $B_6$ intake of less than 0.1 mg. per day, a significant number of babies may develop convulsions, and that 0.2 to 0.3 mg. daily intake should be a conservative recommendation as a minimum requirement. This amount is provided by the usual evaporated-milk formula. For the occasional infant, in which convulsions appear from an inapparent cause, an anomalous condition may be suspected, causing a need for a high $B_6$ intake. In such cases, a therapeutic trial dose of from 5 to 10 mg. of pyridoxine hydrochloride per day is suggested. Frank E. Rice

**628. On the evaluation of artificial feeding.** O. Mellander and B. Vahlquist, Dept. of Medical Biochemistry, Univ. of Gothenburg and Dept. of Pediatries, Univ. of Upsala, Sweden. Am. J. Clin. Nutrition, **5**, 5: 493. 1957.

Some differences between human and cow's-milk protein heretofore not commonly recognized are set forth in detail in this review. Cow's-milk formulas commonly fed to babies not only contain more total protein but considerably more phosphoprotein, which is contained only in the casein fraction. The proteins of cow's milk are digested much faster than the proteins of human milk. This is partly due to the fact that the casein of human milk is much more difficult to hydrolyze completely than the casein of cow's milk. Part of the casein molecule is resistant to the proteolytic enzymes in the alimentary tract. Peptides, particularly those of the phosphorylated type, remain after digestion of the caseins by pepsin and trypsin, more remaining from the digestion of human-milk casein than from the casein of cow's milk. Nutritional significance of the higher phosphopeptide residue from human milk is not clear. The peptides apparently combine with calcium and may thus assist in calcium absorption and the use of calcium in ossification.

Protein-bound carbohydrates are higher in human-milk casein than in cow's-milk casein. The authors suggest the possibility that these components may bear a relation to the type of microbial flora in the intestine, which has been suggested by Gyorgy and coworkers as an important difference in infant-feeding with human milk.

The results of a "field study" are reported, in which observations were made on 402 newborn children, breast-fed for various lengths of time. Breast feeding was supplemented with, or substituted by, a special formula consisting of one part cow's milk, either fresh or dry, one part of water, 5% sugar, and 1% of wheat flour, calculated to contain about 1.7% protein, 1.5% fat, 8% carbohydrate, and 0.4% minerals. Vitamin supplements were given from the age of 1 wk., solid foods at 4 mo.

There was so little difference in response to the various types of feedings that it was considered profitable to compare mainly the "weaned-early" group, A (less than 3 mo. on breast feeding), with the "weaned-late" group, D (breast feeding continued for more than 8 mo.). Babies in the D group gained in weight more rapidly during the first 3 mo., but later more slowly than those in the A group. Observations at from 7 to 8 mo. showed that A-group babies had increased significantly more in height and in number of centers of ossification. Serum calcium levels were about the same in all groups, but serum phosphorus and alkaline phosphatase were significantly lower in the D group. The authors conclude that there was an increased rate of deposition of calcium and phosphorus in infants on cow's-milk formulas. The combined incidence of upper respiratroy infections, otitis, and diarrhea was significantly higher among the babies weaned early (Group A) than among those weaned late (Group D).
Frank E. Rice

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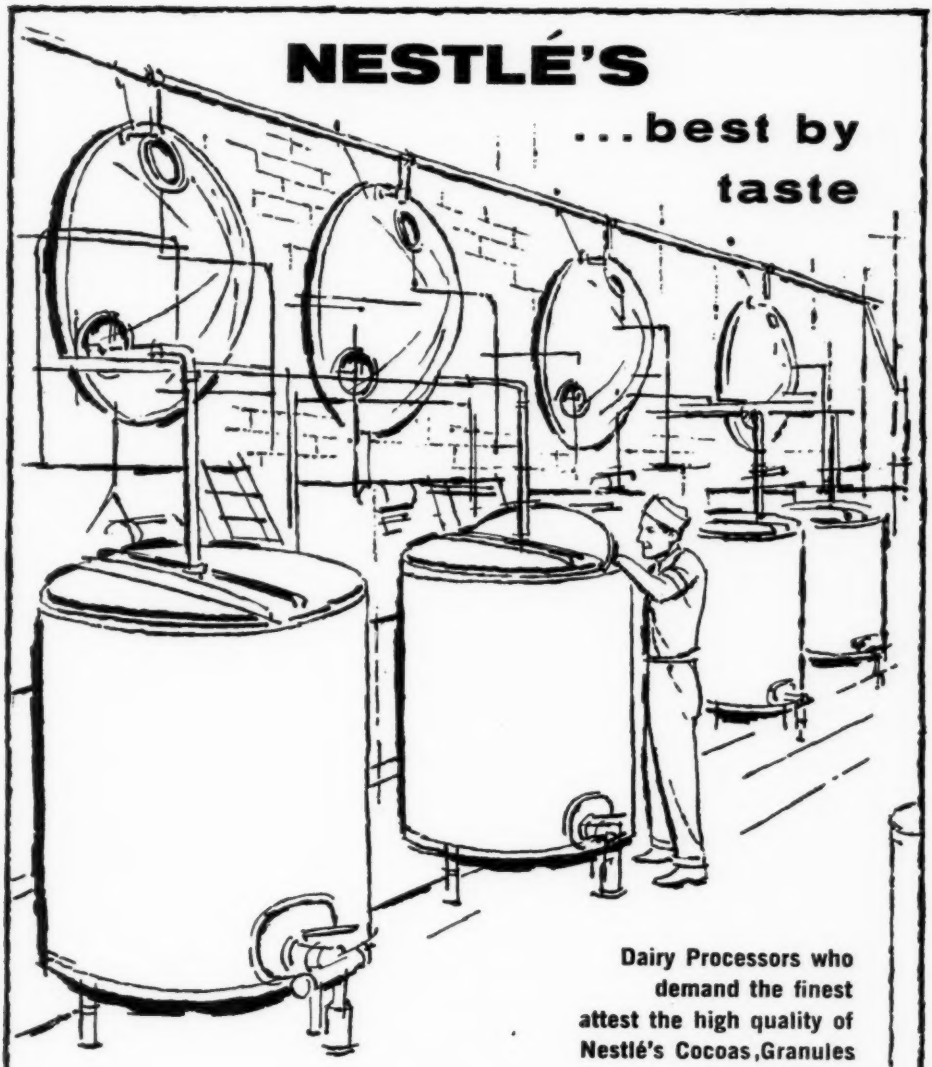

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